7 Cacao

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7.1 Introduction

Cacao, *Theobroma cacao*, a diploid ($2n = 2x = 20$) tropical tree, belongs to the family Sterculiaceae (alternatively Malvaceae *sensu lato*) and order Malvales. It is native to humid topics of the central and northern parts of South America. It is commonly grown in hot, rainy climates between 20°N and 20°S of the equator, with maximum cultivation between 10°N and 10°S (Fig. 1). The plant is the source of chocolate and four intermediary products: cocoa cake, cocoa butter, cocoa powder, and cocoa liquor. In this chapter an overview of *T. cacao*, the methods used for crop improvement, and recent developments in *T. cacao* molecular breeding is presented.

7.1.1 History and Distribution

Cacao has its origin in ancient Central America where the Maya and the Aztecs cultivated it for its seeds (beans), which are used for extracting a drink called *chocolatl*, a precursor to the modern chocolate (Young 1994). Olmec and Mayan civilizations believed that cacao had a divine origin and regarded it as "food of the gods" and thus its scientific name *Theobroma*, (*Theo* meaning "food" and *Broma* meaning "gods") (Coe and Coe 1996). Archaeological evidence suggests that the cacao plantations in the northern and central parts of South America date back to 2,000 years before Spanish contact (Bergmann 1969; Whitlock et al. 2001). The cacao plantations extend from Mexico to Costa Rica, and other locations in South America and the Caribbean (Wood and Lass 1985). The primary center of origin was believed to be the region extending from the forests of the Amazon to the Orinoco and Tabasco in southern Mexico (Whitlock et al. 2001). However, two theories were proposed to explain the origin and domestication of cacao: some studies hypothesized a Central American origin and others a South American origin (Van Hall 1914; Cheesman 1944; Schultes 1984). The hypothesis of Cuatrecasas (1964) of separate simultaneous origins in South and Central America was widely supported (Miranda 1962; Cope 1976; Wood and Lass 1985; Laurent et al, 1994; Whitkus et al. 1998). However, a recent study based on restriction fragment length polymorphism (RFLP) and microsatellite analysis strongly suggests that cacao originated in the Upper Amazon of South America and was later introduced by humans to Central America (Motamayor et al. 2002).

The spread of cacao to other continents was triggered during the post-Colombian era. Hernando Cortez in the sixteenth century was reported to discover the bitter drink used by the Aztecs, and sent the beans and recipes back to Europe (Opeke 1969), where the recipes were refined, and during the nineteenth century technologies were developed that facilitated the roasting and pressing of cacao beans, leading to the creation of eating chocolate, and gained global popularity (Coe and Coe 1996). Cultivation of cacao in other continents was initiated during the colonial era of the eighteenth and nineteenth centuries. It was believed that William Pratt in the 1840s had introduced cacao into a Spanish colony, Fernando Po (presently Bioko in Equatorial Guinea) in Africa, to sustain an interest in cacao drinks, and to produce cacao beans at lower prices from Spanish colonies. From there, cacao was spread to Nigeria, Ghana, Côte d'Ivoire, and other West African countries by trading companies, missionaries, chiefs, and soldiers. Countries such as Sierra Leone, Togo, and Benin embraced cacao cultivation in the early part of the twentieth century (Johns and Gibberd 1951). The early development of the cacao industry in West Africa was entirely due to the initiative and entrepreneurship of the West African peasant farmers (Johns and Gibberd 1951). Owing to good

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Fig. 1. *Theobroma cacao* producing regions of the world (Source: International Cacao Organization)

adoption of the tree and improved trade, West Africa has become a hub for cacao production, contributing 70% of the total world production of cacao beans (Gray 2001).

Currently cacao is cultivated in 6.9 million hectares worldwide with an annual productivity of 3.9 Mt (FAOSTAT data 2006). Over half of the world supply of commercial cacao comes from Côte d'Ivorie (39%), Ghana (19%), and Indonesia (13%) (UNCTAD 2005; Table 1). Brazil, Cameroon, Ecuador, Madagascar, Nigeria, Sri Lanka, and Venezuela export significant amounts. It is also cultivated for export in Columbia, the People's Republic of the Congo, the Democratic Republic of the Congo, Costa Rica, Cuba, the Dominican Republic, Fiji, Gabon, Grenada, Haiti, Jamaica, Malaysia, south central Mexico, Panama, Papua New Guinea, Peru, the Philippines, Sau Tome, Sierra Leone, Togo, Trinidad, and Western Samoa (Soberanis et al. 1999; Duguma et al. 2001; Kraus and Soberenis 2001; Ramirez et al. 2001; Fig. 1). Almost 80% of cacao is grown by smallholder farmers and is often cultivated as a component of complex agro-ecosystems, providing both economic and ecological benefits, like habitat and water conservation, soil stabilization, and carbon sequestration (Wood and Lass 1985; Alves 1990).

Table 1. World production of cacao

Country	Production (metric tons)		
	2002/2003	2003/2004	2004/2005
Côte d'Ivoire	1,367	1,547	1,331
Ghana	498	605	560
Indonesia	413	460	470
Nigeria	178	175	190
Cameroon	152	160	178
Brazil	163	163	171
Ecuador	87	119	110
Malaysia	21	25	26
Other America	172	170	162
Other Africa	39	44	45
Other Asia	64	62	71
Total	4,154	3,530	3,314

Source: LMC International, International Cacao Organization

7.1.2 General Characteristics of Cacao

Cacao is a wide-branching evergreen tree with "cauliflorous" flowers (and later fruits) protruding directly from the woody branches and trunk (Fig. 2). The plant grows to a height of 4–8 m, rarely up to 20 m. The plant produces branches from 1 to 1.5 m height and bears large leaves and produce inflorescence on the trunk and branches. The fruits (commonly referred to as pods) are 10–32 cm long and spherical to cylindrical in shape. The pods are indehiscent and contain 20–60 seeds (referred as beans) arranged in five rows. Unripe pods are

white, green, or red, but turn green, yellow, red, or purple when fully ripened (Reed 1976). Beans from mature pods are removed and fermented to stimulate biochemical activities required for flavor development, but this process destroys seed viability. Usually, cacao seeds are viable for a short time (10–13 weeks) and require up to 50% moisture for germination. In young trees, flowers are produced mainly on the trunk; in adult trees, they emerge all over the plant. An adult tree was reported to produce more than 50,000 flowers per year, less than 5% of which pollinate and only 0.5–2% bear fruits (Alvim 1984). Flowers that were not pollinated within the first 8–10 h after emergence were reported to drop in 24–48 h (Alvim 1984). The potential yields of cacao were reported as 3,375 kg of dry beans per hectare on good plantations, but on-farm productivity of cacao ranges between 29 and 2,000 kg of dry beans per hectare. Trade from dried cacao beans has an annual estimated value exceeding US $3 billion (Gray 2001).

Cacao is commonly cultivated in subtropical dry to wet regions in tropical forest zones (Fig. 1). Commercial cacao plantation is through stem cuttings, buds, or grafts, but direct seed sowings are also done for cultivation purposes. Cacao is often intercropped with other trees of economic value, such as bananas, rubber, oil palm, or coconut. Plants require uniformly high temperatures and thrive well in climates with high humidity and rainfall, but are sensitive to drought (Reed 1976). Plants are shade-tolerant, and thrive in rich, organic, well-drained, moist, deep soils (Reed 1976). Although cacao plants can flower from 2–3 years old, plants are maintained in the vegetative state for 3–5 years by removing flower buds. Subsequently, the plants are allowed to bear flowers and pods (Alvim 1984). Fertilized flowers would take about 4–6 months to mature for harvesting. Although fruits mature throughout the year, usually only two harvests are made. In West Africa, the main harvest season is between September and February, and a second harvest period is between May and June. Matured pods are cut from trees and allowed to mellow on the ground. Then, the pods are cracked, beans are removed, and husks are burned. Beans are fermented in leaf-lined kegs for 2–8 days before drying in the sun. Beans are then bagged and shipped for trade. Further processing includes roasting, crushing, and separating out the kernel, grinding the nibs, and extraction of fat (Fulton 1989).

7.1.3 Botany

T. cacao was the name given to the cacao tree by Linnaeus in the first edition of his *Species Plantarum* published in 1753. The genus *Theobroma*, together with the genera *Herrania*, *Guazuma*, and *Cola*, has been placed in the family Sterculiaceae. However, recent classification based on molecular phylogeny by Angiosperm Phylogeny Group II System suggests *T. cacao* as a species in the subfamily Sterculioideae, of the family Malvaceae *sensu lato* (order Malvales) (Alverson et al. 1999; Angiosperm Phylogeny Group 2003; Baum et al. 2004; Tate et al. 2005). This nomenclature has since been adopted in a number of recent references (Arnold et al. 2003; Sounigo et al. 2005; Sereno et al. 2006).

Phylogeny, Types, and Populations

The natural habitat of the genus *Theobroma* is in the evergreen rain forests of the western hemisphere between 18°N and 15°S, spreading from Mexico to the southern edge of the Amazon forest, and this region is generally considered as the primary center of diversity for cacao (Motamayor et al. 2002). A considerable and useful variation has been recorded in this region through direct observation of trees and also from research on material introduced from this area. Cacao-growing regions around the world are largely centered in important biodiversity hotspots, impacting 13 of the world's most biologically diverse regions (Piasentin and Klare-Repnik 2004).

Cuatrecasas (1964) divided the genus *Theobroma* into six sections consisting of 22 species. *T. cacao* is the only species, which is cultivated widely. The other well-known species in the genus are *T. bicolor* and *T. grandiflorum*. Cacao consists of a large number of morphologically variable populations that are intercrossable with each other. On the basis of the genetic origin, these populations were grouped as autogamous or allogamous (Lanaud et al. 2001). A system of gametophytic–sporophytic self-incompatibility was reported to increase the allogamy of certain cacao populations (Knight and Rogers 1955; Bouharmont 1960; Cope 1962; Glendinning 1962).

Classification of cacao populations into two groups was first proposed by Morris (1882) and named as Criollo and Forastero. From its probable center of origin in the high Amazon region cacao was reported to spread in two main directions,

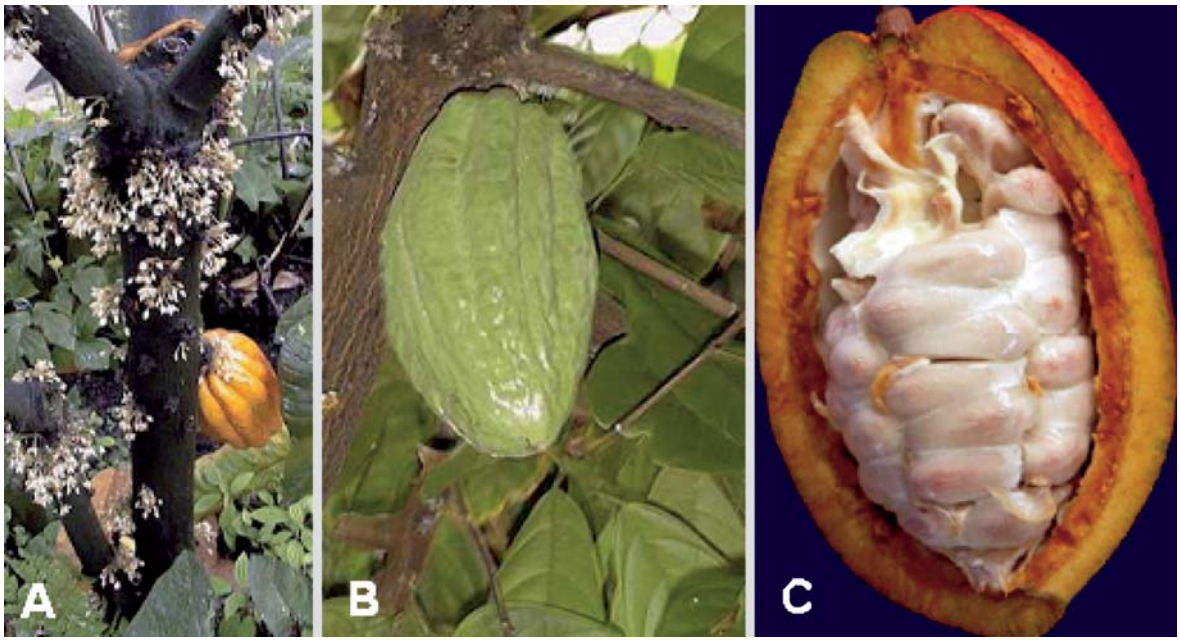

Fig. 2. *T. cacao*: **a** Mature plant, **b** pod, **c** Transverse section of the ripe pod, showing mucilage covered beans (Photos: R. Bhattacharjee)

which resulted in two principal races: (1) the Criollo, grown in Venezuela, Colombia, Ecuador, northern Central America, and Mexico; and (2) the Forastero, cultivated in northern Brazil and Guyana (Cheesman 1944). Pittier (1935) designated Forastero and Criollo as different species and named them as *T. lelocarpum* and *T. cacao*, respectively. However, both these populations being interfertile were designed as a single species, *T. cacao* (Cuatrecasas 1964). Cuatrecasas (1964) proposed two subspecies, *T. cacao* subsp. *cacao* for Criollo and *T. cacao* subsp. *sphaerocarpum* for Forastero, which are similar to the groupings proposed on the basis of the morphogeographic features by Cheesman (1944). A third group was later recognized, namely, Trinitario, consisting of the hybrids between these two subspecies (Dias 2001). The Forastero group covers the majority of world cacao production. The characters of Criollo, Forestero, and Trinitario are presented in Table 2 (Wood and Lass 1985).

Diversity in Cacao Populations

Owing to the outbreading nature of the species, most cacao populations were reported to show a degree of variation for all the morphological characters and even variation at the genetic level (Cuatrecasas 1964). Certain morphological characters of pods and beans were used as the basis to classify cacao into different categories such as varieties, cultivars, types, or populations (Dias 2001). The appearance of the pod, or its morphology, was considered an important character in defining the types and populations in cacao. On the basis this cacao was grouped as follows: (1) Angoleta (deeply ridged, warty, square at the stalk end); (2) Cundeamo (similar to Angoleta but characterized by a bottleneck); (3) Amelonado (smooth, shallow furrows, melon-shaped with a blunt end and slight bottleneck); and (4) Calabacillo (small and nearly spherical). However, taxonomists could not establish a correlation between pod shape and other traits and thus this system was not adopted. The attempts of categorizing cacao genotypes into horticultural races by morphological descriptors (Engels 1986) or isoenzymes (Lanaud 1987) failed owing to large overlaps between the groups.

Characterization of genotypes using molecular markers was believed to provide unambiguous classification and various studies in this direction have generally demonstrated the genetic difference between Upper and Lower Amazon Forasteros, Trinitarios, and Criollos (reviewed in Dias 2001). However, some of these studies have reported difficulties in distinguishing the genotypes/races clearly owing to the occurrence of overlap among the genotype clusters due to

Table 2. Characters and types of populations of *Theobroma cacao*

Criollo

Slender trees, green pods with or without anthocyanin pigmentation. It is regarded as the most anciently cultivated type. The beans ferment quickly and possess excellent flavor, but trees have poor vigor and are extremely susceptible to diseases like bark canker (*Phyophthora* spp.) and Ceratocystis wilt (*Ceratocystis fimbriata*) and are highly susceptible to mirid bug leaf damage. Criollos are subdivided into two geographical groups: Central American Criollos and South American Criollos. Until the middle of the eighteenth century, Criollos dominated the market and accounted for most of the exports to Europe, but at present, only a few pure Criollo types exist. Different types of Criollos have been described by Soria (1970) and Pound (1938) and are as follows:

- *Mexican Criollo*: Occurs only as scattered stands in a few plantings in the state of Chiapas, Mexico. The shape and the size of pods and beans are highly variable, but the beans are white. The color of the pods is between green and red, and the pods have a pointed tip.
- *Pentagona or Lagarto*: In Mexico and Guatemala, within the plantings of Criollo and Trinitario, there are trees that bear pods with very thin, leathery husks; warty with only five angular ridges, red or rarely green in color, and contain seeds with varying shades of purple.
- *Nicaraguan Criollo or Cacao Real*: These populations exist in certain areas of Nicaragua as small populations or isolated groups and are characterized by intense red colored pods with very pronounced bottled neck.
- *Columbian Criollo*: Population based on the color of the pods, which are green and deep purple types, both with a smooth surface and are uniform in their pod characteristics.

Forastero

This is a large group consisting of cultivated populations as well as semiwild and wild types, of which the Amelonado populations are most extensively cultivated.

- *Amelonado*: This population covers a vast cacao area in the state of Bahia, Brazil, and in West Africa (except Cameroon). It is predominantly uniform and homozygous in all its characters. The pod is light green in color with a smooth surface and ridges bottlenecked with a pointed tip that is not very pronounced. The average number of beans per pod is around 40 and the beans are dark purple in color. It is a hardy and productive type, but low in production.
- *Comum*: This variety with typical Amelonado pods was introduced in the late 1700s from the Lower Amazon region as planting material to initiate the cacao industry of Bahia and until recently it covered roughly 90% of the mature plantings.
- *West African Amelonado*: In 1824, a few pods of cacao were successfully transferred by the Portuguese from Brazil across the Atlantic to the island of Sao Tome. By the end of the century, Sao Tome became a major exporter of cacao. The main cacao variety though looks very similar to Comum, but is commonly referred to as West African Amelonado. Toward the end of the 1850s, cacao plants were introduced from Sao Tome to the island of Bioko, Equatorial Guinea. The variety resembled typically as the West African Amelonado.
- *Cacao Nacional*: This is an old variety of Ecuador, but following the incidence of witches'-broom in the 1920s this type was reported to have been virtually wiped out. This variety produces large pale purple beans with distinctive Arriba flavor. The pods are large and green with a rough surface and fairly deep ridges. It is generally considered to be an Amelonado type of cacao.
- *Matina or Ceylan*: This is considered as the Amelonado variety of Central America and is grown commonly in Costa Rica and Mexico. Both probably have a common origin, which may well have been Brazil or Surinam (Soria 1970).
- *Guiana wild Amelonado*: This variety was first discovered and reported by Stahel (1920) in the forests of Surinam toward the western border. Later Myers (1930, 1934) confirmed the general occurrence of this wild cacao in the forests of western Guyana. The pod shape is uniform but typically resembles Amelonados, the seeds are also of the same size and shape as those of Amelonado but their color is bright violet and taste bitter with aromatic pulp. All the trees of this type are heavily infected with witches'-broom.
- *Amazon populations*: These encompass all the populations described and collected by expeditions in the vast Amazon river basin. The pod color is pale to dark green except for some trees with pods having splashes of red and reported from the western extremity of the area where the Napo river extends into Ecuador. Pound (1938) referred to this population as *Criollo de la mantagne* (the native cacao in the forest). The pod shape and husk texture are highly variable, having mostly dark purple cotyledons, but occasionally pale purple beans are reported.

Table 2. (continued)

Trinitario These have features intermediate to those of Criollo, and have descended from an initial cross between Criollo and Forastero (Cheesman 1944, Cuatrecasas 1964). The pod and bean characters are variable. The initial cross resulted in highly vigorous, prolific, hardy trees and these characters continued for a few generations; however in advanced generations the vigor declined but remained higher than that of old Criollo trees. Evidence for this phenomenon has been found in Bioko (Swarbrick et al. 1964), Central America (Soria 1970), and Indonesia. Trinitarios were identified in Trinidad (Pound 1932), Cameroon (Preuss 1901), Samoa, Sri Lanka, Java, Papua New Guinea, and Fiji.
Catongo A population selected for white beans occurs in Brazil, and is propagated mainly through seeds. Catongo types possess seed resembling Criollo, the characters of the pod husk resemble the Amelonado type, and the seed number resembles the Forestero character. The original tree was found in an Amelonado population and was considered to be a mutant. Therefore, the Catongo population was thought to be Forestero type and was described as white-beaned Amelonado.
Djati Roenggo In Indonesia, about 10,000 ha of budded, clonal cacao named as Djati Roenggo has been reported, which has all the pod characters of Criollo types, an exception being that the number of beans per pod is 35, a Forastero or Trinitario character.

the great heterogeneity within the races and the large number of natural hybrids (Lerceteau et al. 1993, 1997; Figueira et al. 1994). However, recent studies utilizing cacao genomics and simple sequence repeat (SSR) markers have paved a way to capture important levels of diversity to understand the origin and for unambiguous classification (Sereno et al. 2006). A set of 15 microsatellites (SSRs) that can detect a high degree of polymorphism have been identified and are proposed as international molecular standards for DNA fingerprinting of *T. cacao* and the development of a unified database of cacao germplasm (Saunders et al. 2001, 2004).

7.1.4 Self-Incompatibility

Self-incompatibility is an efficient common phenomenon in several tropical trees, mostly to avoid inbreeding among them (Bullock 1985), and species in *Theobroma* are no exception. The self-incompatibility system in cacao has been widely described (Knight and Rogers 1955; Cope 1962; Yamada et al. 1982). The incompatibility phenomenon in cacao was reported to be due to the failure of gamete nuclei fusion in the embryo sac. Incompatible mating therefore leads to flower abscission in 72 h after pollination and is characterized by the presence of nonfused nuclei in the ovary. In contrast, Aneja et al. (1994) worked with a self-compatible cacao genotype (IMC 30) and proposed that the self-incompatibility system occurs at pollen germination and gametic fusion stages.

7.1.5 Importance and Uses of Cacao

Cacao originated in lowland rainforests of the Amazon basin but is currently grown throughout the world in the humid tropics and is the main source for the world chocolate industry, which is estimated to exceed US $55 billion per year. A number of products have been derived from the cacao pods and beans. The pod consists of about 42% beans, 2% mucilage, and 56% husk. The bean is composed of the nibs (cotyledons and embryo) and the testa. Cacao contains an alkaloid, theobromine. It is a close structural relative of caffeine. Some references suggest cacao to contain 0.2% caffeine, but this was unfounded. About 3.5% of theobromine is present in fat-free dry cacao beans. It is a stimulant for muscular activity; however, high consumption of theobromine was shown to cause harmful symptoms such as excessive stimulation of kidneys, heart, and smooth muscles. Cacao beans contain about 50% fat that is used in confections and in manufacture of tobacco, soap, and cosmetics. Cacao sweatens (mucilage) is a viscous liquid surrounding the seeds within the pod and provides several by-products (Fig. 3). The mucilage is free of alkaloids and toxic substances, and consists of pectin (5%) and glucose (11%), which are used in the making of jams, jellies, and weight-reducing dietary formula-

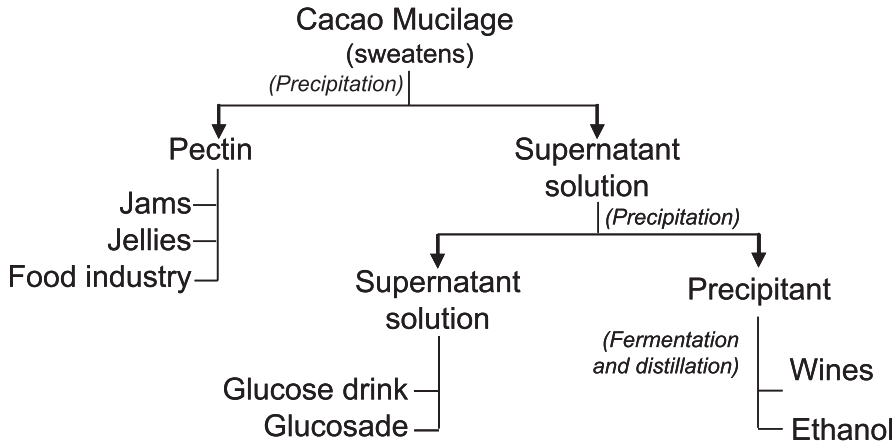

Fig. 3. Industrial derivatives with *T. cacao* mucilage (Source: Wood and Lass, 1985)

tions, and glucose is fermented to produce wines and other alcoholic drinks (Coe and Coe 1996). Cacao is also a folk remedy for burns, cough, dry lips, fever, malaria, rheumatism, snakebite, and wounds. It is reported to be an antiseptic and a diuretic. Emerging studies have shown that cocoa and chocolate are rich in plant antioxidant flavonoids with beneficial cardiovascular properties favoring antioxidant activity, vasodilation and blood pressure reduction, inhibition of platelet activity, and decreased inflammation, and contribute to heart and vascular protection (Engler and Engler 2006; Schroeter et al. 2006). The cacao bean testa (shell) is an important by-product in the chocolate industry and is used as ruminant feed and an organic fertilizer.

7.1.6 Production Constraints

Several diseases caused by fungi, viruses, and nematodes were reported in cacao (Wood and Lass 1985; Bowers 2001), but only a few of them are recognized to be of global importance and most of them are only regionally or locally important. The diseases of global importance are black pod, caused by *Phytophthora* spp. (*P. palmivora*, *P. capsici*, *P. citrophthora*, *P. heveae*, and *P. megakaria*); witches'-broom, caused by *Crinipellis perniciosa*; swollen shoot caused by *Cocoa swollen shoot virus* (CSSV); and monilia pod rot, caused by *Moniliophthora roreri* (Dias 2001). These pathogens contribute to reduction of the potential annual yields by as much as 40%, but the reduction is reported to reach up to 90–100% in cases of diseases such as witches'-broom and monilia pod rot in certain locations (Wood and Lass 1985). CSSV transmitted by mealy bugs is reported to be destructive in West African countries (Muller and Sackey 2005). Diseases such as cacao wilt (Ceratocystis blight), caused by *Ceratocystis fimbriata*, Verticillium wilt, caused by *Verticillium dahliae*, and pink disease, caused by *Corticium salmonicolor*, are of local occurrence, although they are sometimes of relative economic importance. Wood and Lass (1985) have given detailed information on symptoms, taxonomy, origin, dissemination, transmission, and disease control.

Chemical and nonchemical management strategies to control important cacao diseases were found to be ineffective owing to high costs and difficulties in application. The combined practice of phytosanitary pruning with adequate fungicide application is widely used for the control of most diseases (Wood and Lass 1985; Dias 2001; Krauss and Soberanis 2001). But such practices are expensive and not ecofriendly. Extensive efforts are being made to replace susceptible cultivars with durable resistant cultivars. However, progress in this direction is slow owing to limited sources of genetic resistance in cacao against most of these pests and pathogens (Eskes and Lanaud 1997), but recent advances in cacao genetics and molecular techniques are enhancing this process (Eskes and Lanaud 1997; Bennett 2003; Risterucci et al. 2003; Clement et al. 2004). Furthermore, protocols for transformation and regeneration of cacao plants have been established and these can contribute to the development of transgenic resistance against major pathogens (Maximova et al. 2005; Sec. 7.6).

7.2 Cacao Breeding

In the case of cacao, it has been estimated that about 30% of the total cultivated area is planted with selected varieties (Paulin and Eskes 1995; Eskes 2001), consisting mostly of mixtures of biparental crosses (hybrids) between local and introduced clones. The remaining 70% of cultivation has been with traditional populations such as Trinitario, Amelonado, F3 Amazon, and open-pollinated populations derived from selected hybrid varieties. Studies have shown that farmers tend to increasingly use seeds from their own preferred trees (Opoku et al. 2006), which is expected to result in partial inbreeding and a narrow genetic base of cultivated cacao, making it vulnerable to various diseases (Bennet 2003), and loss of vigor and yielding capacity (INGENIC 1995, 1999). The past and on-going cacao improvement programs involve germplasm management, characterization, and evaluation; development of breeding tools (early screening methods, application of molecular tools); genetic studies; creation and selection of new varieties (on-farm varietal trials); and multiplication and distribution of new planting materials (INGENIC 1999; Eskes 2001). The major breeding methods employed in cacao are described next.

7.2.1 Clone Selection

Cacao breeding started in the 1920s when clones were selected in commercial plantations. Since then, clone selection has been the major breeding method carried out in most of the cacao-producing countries. These clones were further used to establish collections of local material for use in making crosses with introduced genotypes and to obtain new hybrid cultivars. In some cases, clones were selected on the basis of higher yield and quality and were used as commercial varieties, such as Trinitario clones (ICS, DR). These clones are still being used as cultivars in Trinidad and Indonesia (Thévenin et al. 2004). Since 1970s, new clones have been selected mainly to obtain rapid progress for resistance to devastating diseases such as vascular streak dieback in Southeast Asia and to witches'-broom (Trinidad selected hybrid clones) in Trinidad, and more recently in Brazil. Recently, large-scale selections of commercial clones with high resistance to black pod have also been started (Eskes et al. 1998; Efron 2000).

7.2.2 Hybrid Selection

Heterosis observed in individuals obtained from crosses between genetically distinct genotypes is the basis for hybrid selection. In worldwide cacao breeding programs between the 1950s and the 1990s, selection of new hybrid cultivars was the main activity. Crosses were generally made using local and introduced clones available in the germplasm collections as parents. Looking into the progress made with the introduction of these hybrids for agronomic traits such as early production (precocity), yield capacity, and vigor, large-scale utilization of hybrids started in cacao breeding. However, this method did not obtain good disease resistance.

Significant improvement in cacao productivity with the development of superior hybrids was reported from Brazil, and there is a consensus occurs among experts that hybridization is and will remain the main breeding method for cacao (Gotsch 1997). It was demonstrated by Dias et al. (1998) that in a large field trial there is a need for using cacao tree hybrids along with traditional local cultivars. Generally cacao tree hybrids show wide adaptability, low interaction with time, and better performance for yield and its components along with some resistance to diseases, in comparison to unimproved traditional local cultivars (Dias and Kageyama 1995). However, the major drawback in cacao hybridization is its empirical use of geographic divergence as an indicator for genetic divergence and this is still a common practice worldwide, though in many instances the positive correlation between geographic and genetic divergence does not exist (Dias et al. 1997).

The other frequently adopted criterion in cacao breeding is complementation of traits of interest, mainly to get rid of the deficiencies in each parent. Crosses are conducted randomly, primarily when information about the parental clones is not available. Because the yield ability of the clones per se is not associated with their performance as progenitors, combining ability tests are used to overcome part of the randomness of the hybridization process (Dias et al. 2003). However, according to Bos and Sparnaaij (1993), the use of a combining ability test in a diallel crossing scheme to study a complex trait like yield has lost its appeal because of the restricted resources in terms of land and labor which limit the number of parents that can be tested, and the almost consistent conclusion that variance due to general combin-

ing ability effects exceeds the variance due to specific combining ability. Since the long juvenile period of cacao lasts from 3 to 5 years and a single selection cycle might last one decade, new predictive tools provided by quantitative and molecular genetics must be used more intensively in cacao breeding programs.

Most of the breeding efforts in cacao have been concentrated toward selection based on seed yield and disease resistance. Very little is known about the available diversity for fat content within *T. cacao* for this trait (Kennedy et al. 1987; Lockwood and Yin 1993). There is also little knowledge available about the mode of inheritance of this trait and the effect of environmental factors on fat content.

7.2.3 Recurrent Selection and Prebreeding

Generally the traditional procedure of selecting hybrids does not lead to continuous genetic progress, therefore, breeders use successive breeding cycles to increase the frequency of favorable alleles in parental populations (Toxopeus 1972; Kennedy et al. 1987). Recurrent selection procedure is a robust method that exploits general and specific combining ability effectively, predominantly for most traits in cacao, mainly when populations are based on genetically related individuals, such as Lower Amazon types (Baudouin et al. 1997). In Côte d'Ivoire, a recurrent selection program was initiated using two base populations (Clement et al. 1994). Two cycles of selection were proposed in order to increase the frequency of favorable alleles for traits with relatively high heritability (e.g., disease resistance, self-compatibility, and pod index) in the base population. Countries such as Brazil, Ghana, and Malaysia also initiated this program to increase the number of favorable alleles in the locally adapted germplasm.

Prebreeding is a specific form of recurrent selection of improving genetically distinct base populations for specific traits in large germplasm collections before distributing the materials to users. The Cocoa Research Unit of the University of the West Indies, managing the International Cocoa Genebank in Trinidad, has started a prebreeding program with emphasis on disease resistance (Iwaro and Butler 2002).

Most of the ongoing breeding programs are concentrating on developing disease and pest resistance, especially in those cases where suitable screening methods are available. In addition to breeding for yield, breeders are selecting for more efficient and smaller trees that can be easily managed by farmers. Quality, such as flavor traits, is becoming a major selection criterion for breeding (INGENIC 1999)

7.3 Genetic Linkage and Gene Mapping

Cacao is a diploid with a chromosome number, $2n = 20$, and a small genome (0.4 pg/C) (Lannaud et al. 1992), about 2.8 times the size of that of *Arabidopsis thaliana* (L.) Heynh. (Couch et al. 1993). Lannaud et al. (1995) published the first linkage map of cacao developed from an F_1 cross of an Amazonian Forastero clone UPA402 with a Trinitario selection, UF676, from Costa Rica. UPA402 in turn was produced from sib-mating of two Ecuadorian Forastero clones, IMC60 and Na34 (Lannaud et al. 1995). The initial map from 100 individual trees was developed on the basis of five isozyme loci, four functional genes, 55 RFLPs of genomic DNA, and 28 randomly amplified polymorphic DNAs, resulting in ten linkage groups, which correspond to the number of chromosome pairs in cacao. The recombination-based genome length map thus obtained was reported to cover 759 cM (Lanaud et al. 1995).

Risterucci et al. (2000) saturated the map with additional markers (424 in total) and produced the first high-density linkage map of cacao using 81 additional trees. The high-density map of Risterucci et al. (2000) covered 885.4 cM. Both the genetic maps resulted in ten linkage groups having good agreement for marker alignment (Lannaud et al. 1995; Risterucci et al. 2000). Pugh et al. (2004) reported a genetic map using the same parental populations used by Lanaud et al. (1995) and 135 progenies. This map included the codominant markers from previous maps and 201 new SSR (microsatellite) markers and 16 resistance gene analog based markers (Lanaud et al. 2004). A total of 465 markers were used to develop a map that has the genome length of 782.8 cM (Pugh et al. 2004). A slight reduction in distance compared with the high-density map of Risterucci et al. (2000) was attributed to amplified fragment length polymorphism marker bias toward AT-rich, heterochromatin sites located near the centromeres and telomeres (Pugh et al. 2004). However, the colinearity was reported to be very high between the two maps, and genetic distances

between loci common to both maps were generally of the same magnitude (Risterucci et al. 2000; Pugh et al. 2004).

Brown et al. (2005) used 146 progenies and 204 codominant markers (174 SSR markers identified by Lanaud et al. 1999b; Risterucci et al. 2000; Pugh et al. 2004; and 18 SSR markers and eight resistance gene homologs from Kuhn et al. 2003; and four WRKY genes from Borrone et al. 2004) to developed a map using the Kosambi mapping function and JoinMap 3.0 (van Ooijen and Voorrips 2001). Ten linkage groups were reported from this study, corresponding to the ten chromosomes of cacao with a total genome length of 671.9 cM. This map was 213.5 cM shorter than the high-density linkage map (885.4 cM) produced by Risterucci et al. (2000) and 110.9 cM shorter than the F_1 codominant map of Pugh et al. (2004).

The first genetic map was used for the identification of the quantitative trait loci (QTL) for resistance to black pod disease (Lanaud et al. 1999a). Subsequently, QTLs for yield and yield-related agronomic traits and resistance to black pod disease caused by *Phytophthora pulmivora* were reported by Crouzillat et al. (2000a, b) and Flament et al. (2001), respectively. The QTLs identified were reported to account for moderate (17%) to relatively high (48%) levels of resistance to black pod disease. However, Flament et al. (2001) observed that none of the QTLs were common across the three different measurements for resistance, and found results from artificial inoculation data to be poorly related to results based on field resistance, as did Lanaud et al. (1999a). A three-way test cross was used by Clement et al. (2003a, b) with two Trinitario clones (DR1 and S52) and one Upper Amazon clone (IMC78) crossed onto one homozygous Lower Amazon clone (Catongo) for map construction and QTL colocalization for yield and yield-related traits, and for black pod resistance. This effort was successful in mapping several yield and yield-related traits with good reliability and commonality among correlated traits. Resistance QTLs for black pod were found in DR1 and IMC78 in similar regions of chromosome 4 to those reported earlier by Flament et al. 2001.

Brown et al. (2005) used an F_2 population (derived by selfing TSH516, a clone from a cross of Sca6 with ICS1, selected in Trinidad) segregating for resistance to witches'-broom to construct a map of *T. cacao*. Quieroz et al. (2003) used earlier a subset of an F_2 population comprising 82 progenies and mapped mostly dominant markers, and identified a putative QTL conferring resistance to witches'-broom on the map containing 25 linkage groups.

MapQTL 4.0 (van Ooijen and Maliepaard 1996) was used to locate the putative QTLs for phenotypic traits, using simple interval mapping, followed by the multiple QTL mapping procedure of Jansen (1993). Two major QTLs controlling resistance to witches'-broom were detected, one located on linkage group 9 with a very high logarithm of odds score (10.55) explaining nearly 51% of the variance for the trait, and the other located on linkage group 1 with a logarithm of odds score of 3.38 explaining 6.7% of the variance.

The availability of the F_2 map corresponding to the specific chromosome number will allow better comparisons of the cacao genome with that of many other annual crops, in which maps are more often made from F_2 or recombinant inbred populations. Further, development of anchor markers with other important members of the genus *Theobroma* L. such as *T. grandiflorum* (Willd. ex Spreng.) Schum (de Sousa Silva et al. 2001), and with members of the Malvaceae family such as *Gossypium hirsutum* L. and other dicotyledons would be useful for genomic comparisons and for gene expression studies.

The established molecular maps of the cacao genome are now providing a rational direction for systematic breeding programs, most importantly to broaden the genetic base of cultivated trees as well as to characterize the existing *T. cacao* germplasm collections (Bennett 2003; Clement et al. 2004; Sereno et al. 2006).

7.4 Marker-Assisted Selection

QTL analysis has a greater potential for identification of markers for marker-assisted breeding, especially for incorporation of disease resistance. There have been several studies that showed QTLs associated with traits of interest in *Theobroma* (Crouzillat et al. 1996, 2000a, b, 2001). Major QTLs were identified for resistance to *C. perniciosa* (agent of witches'-broom) (Quieroz et al. 2003) and for yield traits such as pod weight (Clement et al. 2003a, b). The prospect of progress from identification of QTLs to the isolation of gene(s) by map-based cloning has not yet been applied in cacao. Such an approach requires a very large population to accumulate a suitable number of recombinant individuals (around 1,000), which is not

only expensive in terms of time and resources but is also extremely difficult in the case of cacao (Wilkinson 2000). The bacterial artificial chromosome (BAC) library developed recently by Clement et al. (2004) can be a valuable resource for cloning genes corresponding to some important QTLs.

7.5 Application of Genomic Tools and Gene Discovery

Relatively less attention has been directed toward gene discovery and expression studies in cacao. Recently, a variety of genetic and genomic resources were applied to identify genes involved in disease resistance using a high-density linkage map, and the identification of disease-associated QTLs (Bennett 2003), expressed sequence tags (ESTs)/array technologies (Jones et al. 2002; Verica et al. 2004), and BAC libraries (Clement et al. 2004). Resistance gene analogs and defense gene analogs were isolated from cacao genomic DNA using degenerate primers designed from conserved domains of several plant resistance defense genes (Kuhn et al. 2003; Lanaud et al. 2004). Most of the resistance and defense gene analogs were mapped in segregating populations and QTLs for resistance to *Phytophthora* spp. were identified (Crouzillat et al. 2000b; Clement et al. 2003a; Resterucci et al. 2003). The cacao BAC library was constructed with 36,864 clones from the genotype Scavina-6 (Clement et al. 2004), and was used to identify QTLs for resistance to *Phytophthora*, witches'-broom, and black pod disease. More than 5,500 ESTs have been sequenced, from which a set of 1,380 unique gene sequences have been derived from beans and leaf complementary DNA (cDNA) libraries (Verica et al. 2004). These could be used to identify differentially expressed sequences for both bean and leaf traits. Microarrays have been constructed and used in demonstration experiments to evaluate genotype and tissue-specific gene expression using the publicly available sequences from the *T. cacao* unique gene set, and the expression was validated by real-time polymerase chain reaction (Jones et al. 2002). Verica et al. (2004) used subtractive hybridization of cDNA libraries, macroarray hybridization analysis, and high-throughput DNA sequencing to identify cacao genes in the plants treated with inducers of defense response and identified a unigene set of 1,256 members, including 330 members representing genes induced during the defense response. Unigene sets of Jones et al. (2002) and Verica et al. (2004) were shown to have 10% of the sequences overlapping, giving rise to a combined unigene set of about 2,500 sequences (Verica et al. 2004). These sequences are a potentially valuable resource for identifying novel defense genes. In addition, candidate defense genes can be used to search for colocalization with QTLs associated with resistance to cacao diseases and also are valuable when used in combination with the germplasm resources to evaluate the underlying molecular basis of disease response as well as bean quality traits that contribute to bean yield, composition, and flavor (Bennett 2003).

7.6 Genetic Transformation

The application of tissue culture and genetic transformation complemented by traditional plant breeding programs plays an important role in enhancing productivity and bean supply (Eskes and Lanaud 1997; Wilkinson 2000). This approach opens up an opportunity to incorporate novel sources of resistance genes or any other valuable traits into the genome and then can be used in breeding programs (Hansen and Wright 1999; Persley 2000). While these approaches have been utilized for many plant species, they have been limited in cacao because of several technical difficulties posed by the species. The first cacao transformation attempt resulting in transformed callus cells was by Sain et al. (1994). Subsequently, Maximova et al. (2003) reported the development of an efficient genetic transformation system for cacao using somatic embryogenesis as a regeneration system, coupled with *Agrobacterium tumefaciens* cocultivation that resulted in transgenic plants. The transformed plants were grown to maturity and segregation and expression of the transgenes in the subsequent T_1 generation was demonstrated (Maximova et al. 2003). This technology was used to develop the world's first transgenic cacao overexpressing class I chitinase gene (*TcChi1*) isolated from cacao to enhance resistance against *Colletotrichum gloeosporioides* (Maximova et al. 2005). It was reported that the transgenic line has up to a six-fold increase of endochitinase activity compared with nontransgenic and transgenic control plants (Maximova et al. 2005). This study demonstrated for the first time the utility of the transformation system as

a tool for gene functional analysis and the potential utility of the cacao chitinase gene for increasing fungal pathogen resistance in cacao and also the scope for incorporating resistance to other fungal and viral pathogens, and insect pests. However, genetically modified cacao material has not yet been released and the scope of transgenic cacao will depend upon economic, social, environmental, and political factors of the introducing country. Nonetheless, the efficient tissue culture system on its own contributes to clonal propagation of elite germplasm.

7.7 Future Prospects

The cacao plant originated in the Amazon basin of South America has emerged as one of the most successful commercial tree crops. Despite its continuing importance as a chief crop supplying raw material for the multibillion-dollar confectionary industry, research on genetic improvement of *T. cacao* has been slow. However, efforts for systematic genetic improvement of *T. cacao* have gained pace during the past decade, leading to development of a high-density linkage map, SSR markers, and even efficient tissue culture and transformation protocols.

Several new technologies of recent emergence have dramatically increased the genetic knowledge on cacao and provided tools to accelerate the breeding/selection programs to develop elite cacao varieties. The established molecular maps of the cacao genome are contributing to a rational direction for systematic breeding programs, most importantly to broaden the genetic base of cultivated trees as well as to characterize the existing cacao germplasm collections, leading toward unambiguous classification of germplasm. There is a need to improve the resolution and accuracy of QTL analysis in cacao. This requires large mapping populations across several geographically dispersed sites and repetition of phenotypic scoring over several years to ensure stability of the identified QTLs. It is necessary to understand the components of cacao crop management to grow sustainable cacao by reducing the impact of diseases such as black pod, witches'-broom, frosty pod rot, and swollen shoot. Private and public partnerships for research for genetic improvement of cacao can boost the development of much needed technologies to enhance cacao production and quality. As stated by Bennett (2003), in the coming years, cacao is likely to become a model for other tree crops for being transformed through genome-based breeding.

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