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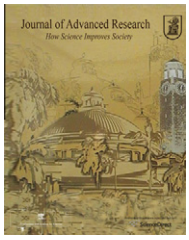

REVIEW ARTICLE

A review of three major fungal diseases of *Coffea arabica* L. in the rainforests of Ethiopia and progress in breeding for resistance in Kenya

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Abstract In a review of their own research the authors summarize incidences and distributions of the most important fungal diseases in Ethiopia and progress in breeding for resistance. Ethiopia, as the centre of origin for *Coffea arabica*, hosts a large diversity of germplasm. The incidences of diseases are based on observations in the montane rainforests of the southeast (Harenna) and southwest (Bonga, Berhane-Kontir, Yayu) of Ethiopia. Major diseases are Coffee Leaf Rust (CLR), *Hemileia vastatrix*; Coffee Berry Disease (CBD), *Colletotrichum kahawae* and Coffee Wilt Disease (CWD), *Gibberella xylarioides* (*Fusarium xylarioides*). CLR incidences in Ethiopia were present in all regions with highs between January and March and lows between June and October. CBD was present mostly in Bonga (40.0%) and Yayu (26.3%), but less frequent in Harenna (18.6%) and Berhane-Kontir (6.0%). CWD as a recently developed disease in Arabica coffee could be detected ranging from 2.4% in Berhane-Kontir to 16.9% in Yayu. CLR has been a serious constraint in all production countries since it became prominent in Ceylon in the late 19th century after leaf infection defoliation affects plants. CBD was first observed in Kenya in 1922. The disease is currently confined to the African continent in all countries that grow Arabica coffee. In the mid-1990s in the Democratic Republic of Congo, Uganda and Tanzania a resurgence of CWD in Robusta coffee and in Ethiopia in Arabica coffee occurred. Over the last 40 years breeding activities have been car-

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ried out to combat CLR, CBD and CWD. Breeding for resistance against CLR in Arabica coffee has successfully utilized single or combinations of major genes designated as S_H genes. Major gene resistance has also been deployed in breeding for resistance against CBD, whereas in the case of CWD, selections of tolerant Arabica accessions are being pursued from local landraces in Ethiopia.

Introduction

The following review of coffee diseases comprises first a description of three major fungal pathogens: Coffee Leaf Rust (CLR), *Hemileia vastatrix*, Coffee Berry Disease (CBD), *Colletotrichum kahawae* and Coffee Wilt Disease (CWD), *Gibberella xylarioides* (*Fusarium xylarioides*) including the historical occurrence, distribution, symptomatology, biology of the pathogens and their economic importance [1]. Control measures of such immense disease agents are essential; therefore, in the second part of the review sustainable efforts in breeding for resistance are described. The presented data are based on the experimental experiences and activities of both authors and their working teams in Ethiopia and Kenya.

The host, *Coffea arabica* L.

The genus *Coffea* is endemic to Africa and a number of species are described in West, Central and East Africa. Due to disease constraints and other factors such as yield, quality and growth habits, only two species are nowadays commercially grown worldwide, namely *C. canephora* (Robusta) in lowlands and *C. arabica* (Arabica) in highlands. Arabica coffee is grown in altitude ranges between 1400 and 1800 m and is cultivated under shade. This species originated from the province of Kaffa in Ethiopia and was distributed by Yemen traders all over the world during the 15th century. Today, in a few remaining rainforests of southwest and southeast Ethiopia, coffee grows as an understory shrub in a large diversity of shade trees, shrubs and annual plants and has maintained its own genetic diversity as a natural gene-bank. But even this natural resource is not free of diseases. It continues, however, to survive all attacks by pathogens and pests in a unique way under natural conditions. Therefore the description and occurrence of diseases will concentrate on experiences in the montane rainforests of Ethiopia.

Field sites in Ethiopia

Investigations of the occurrence of diseases were carried out in four different rainforest regions of the southeast (Harenna in the Bale Mountains) and southwest (Bonga, Berhane-Kontir and Yayu) of Ethiopia. Details of the field sites are shown in Table 1.

The pathogens

The disease frequency of indigenous coffee in the four major rainforest areas in 2005 is taken to represent the situation in general during the investigation period of 2003–2008 (Fig. 1).

Coffee Leaf Rust (Plate 1A), *H. vastatrix*

Coffee Leaf Rust (CLR) is one of the most important diseases of *C. arabica* in the world. It devastated Arabica coffee plantations in Ceylon at the end of the 19th century and was responsible for its replacement with tea plantations. Despite effective fungicides and resistant varieties developed to control rust, yield reductions of 20% or more in various countries are still caused by the pathogen [2]. In Brazil, losses have been estimated to be about 30% and an annual loss of about 4500 tons of coffee was estimated in Kenya in the 1960s. The pathogen prefers a temperature range of 20–28 °C, needs a leaf wetness period only during spore germination and penetrates with the germination hyphae into the stomata of the host. The fungus tolerates longer seasons without rainfall and spores are wind-borne, only attacking leaves and needs no other host

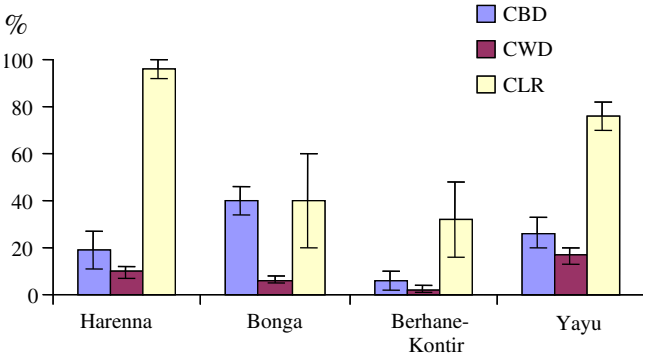


Fig. 1 Disease incidence in indigenous coffee 2005 [24].

Table 1 Field sites of indigenous coffee of southeast and southwest Ethiopia.

Code, habitat and plots	Latitude (N)	Longitude (E)	Altitude (m a.s.l.)	Annual precipitation	
				Mean (mm)	CV[a] (%)
I. Harenna 1, 2, 3	6°30′	39°45′	1580–1610	850 (20)	26
II. Bonga 1, 2, 3	7°30′	36°30′	1750	1700 (44)	16
III. Berhane-Kontir 2, 3	7°05′	35°30′	1200–1320	2100 (25)	13
IV. Yayu 1, 2	8°20′	35°50′	1530–1600	1800 (32)	11

[a] CV = coefficient of variation [%].

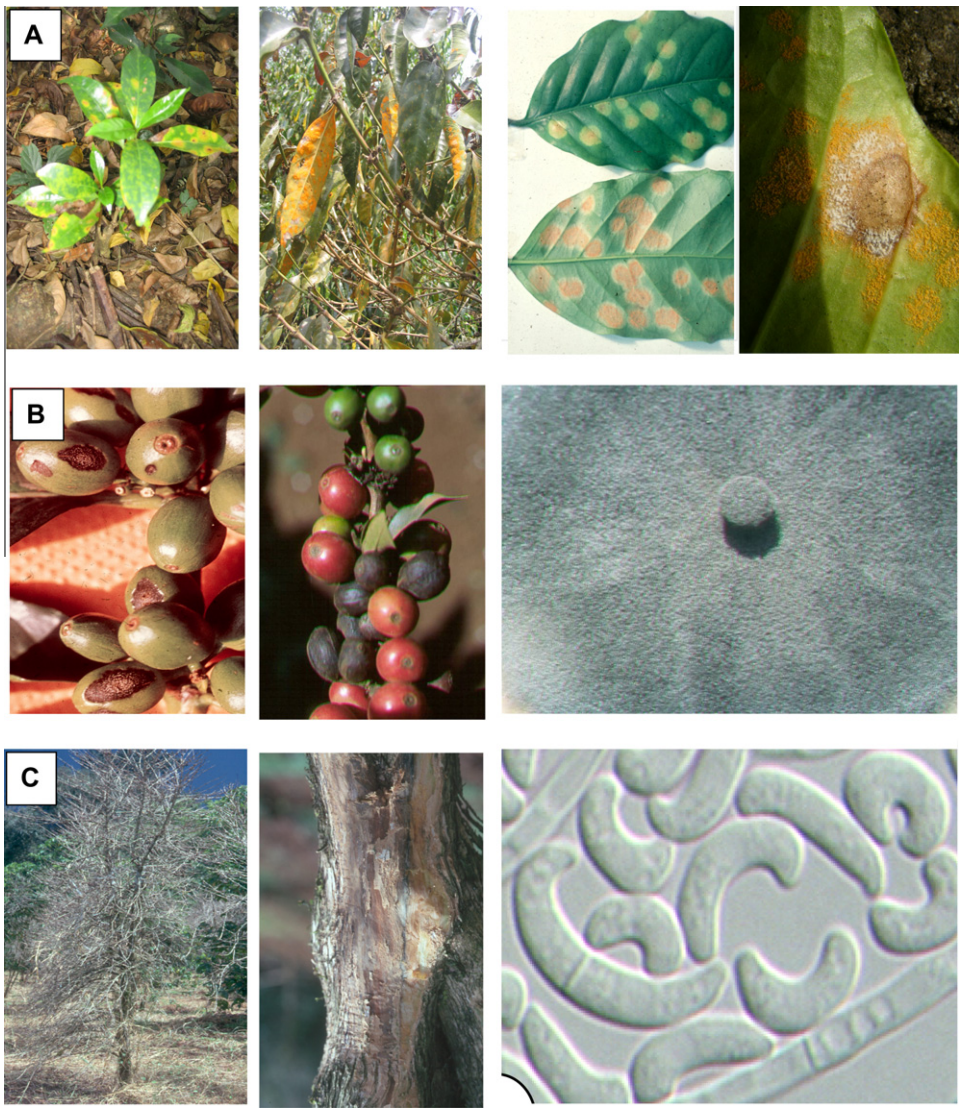


Plate 1 Symptoms of fungal diseases of coffee. (A) Coffee Leaf Rust: on seedlings, older leaves, upper and lower site of the leaf and hyperparasitized by *Verticillium lecanii*. (B) Coffee Berry Disease: on green and mummified berries, mycelium colour on Malt-Extract Agar. (C) Coffee Wilt Disease: dead tree, brownish vascular system on stem, conidia of the imperfect stage *Fusarium xylarioides*.

for completing the life cycle. Due to the fact that coffee is a perennial host with green leaves all through the year, the pathogen produces only uredinio- and teliospores with basidiospores. Coffee grown in lower altitudes is more predisposed to the disease and suffers more attacks. A heavy infestation of leaves not only reduces the assimilation area but also results in a complete defoliation diminishing the next year's crop tremendously.

CLR was first reported in Ethiopia in 1934 [3], but the disease had existed for a long time in other countries without causing epidemics or eradications of certain varieties of *C. arabica*. The long-term coexistence of coffee and rust coupled with the high genetic diversity of coffee populations and a high level of horizontal resistance might have kept the rust at low levels [4]. Other factors such as the low average productivity associated with shade and the existence of biological agents such as the hyperparasite *Verticillium lecanii*, were also believed to play an important role in maintaining CLR at low levels.

A large number of urediniospore samples were collected in the Ethiopian rainforests and identification was carried out during 2003/04 in the Institute of Botany, Tübingen University [5]. Measurements of urediniospores of CLR from the indigenous coffee population revealed detailed data with typical sizes for the species of *H. vastatrix* and had spore dimensions between $29.7\mu \times 18.9$ (minimum) and $34.5\mu \times 23.7$ (maximum). These spore sizes could be compared with those identified in highly susceptible Ethiopian selections such as Arba, Guga and Harrar and others from Indonesia and Colombia. The results showed that measurements were to a large extent identical and confirmed the presence of the species *H. vastatrix* (Table 2). The identification proof of the species *H. vastatrix* by morphological characteristics was assisted by scanning electron microscopic photos of rust sori and urediniospores [5]. A typical sorus extruding from a stoma on the lower side of the leaves had 15–25 lemon-shaped one-celled urediniospores (Fig. 2).

Table 2 Sizes of urediniospores of *Hemileia* spp.

Location	Coll. date	Length	Width	Variations
I. Harenna 1	8.2004	33.7	22.1	31–36 × 21–23
II. Bonga 1	5.2004	32.7	23.7	29–36 × 21–26
II. Bonga 2	11.2003	30.9	20.4	30–33 × 20–22
II. Bonga 2	11.2003	30.5	21.2	29–32 × 20–23
II. Bonga 2	11.2003	30.1	19.8	28–31 × 18–21
II. Bonga 2	11.2003	30.0	20.5	28–32 × 20–22
II. Bonga 2	11.2003	30.9	20.4	30–33 × 20–22
II. Bonga 3	1.2004	30.3	18.9	29–33 × 18–20
II. Bonga 3	5.2004	31.8	23.3	27–37 × 20–26
III. Berhane-Kontir 2	1.2004	33.2	19.5	30–36 × 18–21
III. Berhane-Kontir 2	5.2004	29.7	21.9	26–33 × 17–25
III. Berhane-Kontir 3	1.2004	32.1	19.9	30–34 × 19–21
IV. Yayu 1	11.2003	30.4	20.4	29–32 × 20–22
IV. Yayu 1	5.2004	30.3	22.7	27–34 × 20–25
IV. Yayu 2	11.2003	31.2	19.8	30–33 × 19–20
IV. Yayu 2	5.2004	34.4	21.3	31–38 × 19–23
Mean	2003/04	31.61	21.19	26–40 × 17–26

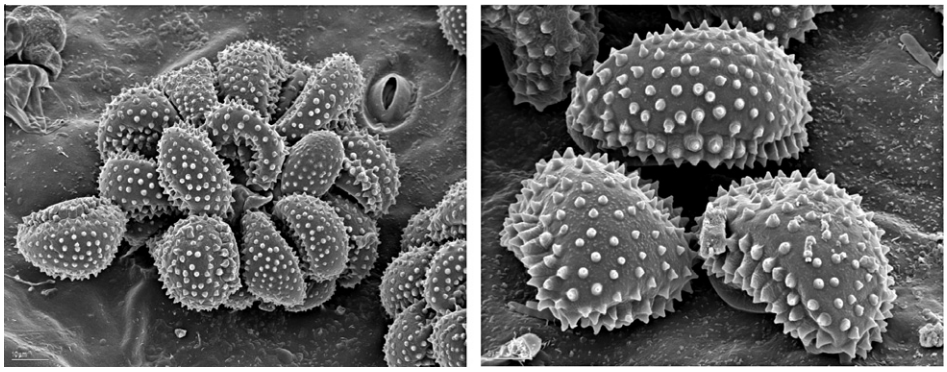


Fig. 2 Urediniosorus and urediniospores of *Hemileia vastatrix*.

There was little emphasis on race-typing of Ethiopian rust samples until the beginning of the 1980s and the 1990s, when the Institute of Biodiversity Conservation (IBC, formerly gene-bank) included coffee in their conservation system. Wondimu et al. [6] observed that race III was the most frequent in forest coffee and race II in other areas. Other races were I, X and XV. In 2005 the first race-typing of CLR collections of indigenous coffee was carried out at the Centre of Coffee Leaf Rust Research (CIFC) in Oeiras, Portugal using their differentials (Varzea, personal communication). In this recent study the race specification identified race II at Berhane-Kontir and race III and X in Bonga with corresponding virulence genes v 1, 4 and 5 [7].

CLR assessments in the rainforests of Ethiopia revealed its presence in all fields differing in incidence with time (season) and location. A significantly ($P < 0.001$) high rust incidence of 31.1% was recorded, for instance, in 2008 at Yayu, followed by Berhane-Kontir (21.4%) and Bonga (7.9%) in forest coffee populations. Rust incidences were consistently highest in Yayu, lower in Berhane-Kontir and lowest in Bonga forests during all seasons. The occurrence of rust in the forest coffee populations varied significantly from season to season ($P < 0.001$). Higher rust incidences were found in January (29.6%) and April (22.7%), while lower incidences were observed in July (13.9%) and October (14.3%).

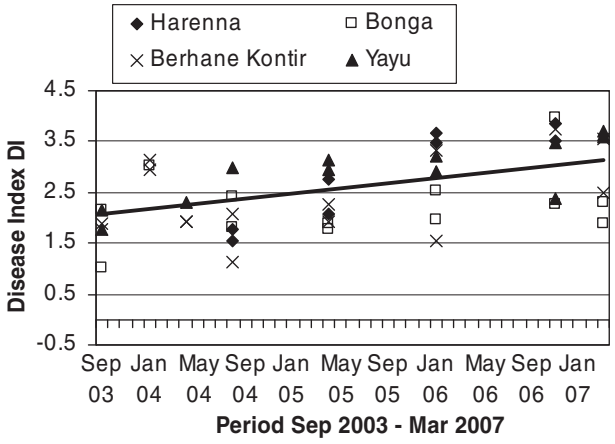


Fig. 3 CLR severity during 2003 and 2007 in indigenous coffee populations of Ethiopia [24].

Comparing rust occurrence during the complete period of the surveys from 2003 to 2007 a slight increase of the disease could be observed in the wild coffee population (Fig. 3).

The effect of shade on the occurrence of CLR could be shown in nursery experiments at the Jimma Agricultural Research Centre (JARC). All young coffee trees grown under the shade were infected more seriously with rust than in the non-shaded sites. Comparing coffee from the different forest regions, the material from Bonga seemed to be more tolerant to rust than others [7].

Coffee Berry Disease (Plate 1B), *C. kahawae*

CBD was first detected in 1922 in Kenya around Mt. Elgon, west of the Rift Valley [8,9]. Soon after detecting the disease, losses of up to 75% were reported. This brought the coffee cultivation west of the Rift Valley to a near end and tea plantations became predominant in the region. The dry Rift Valley stopped the spread to the major coffee-growing areas in the highlands of the Central Province for a long time. In 1951 a first appearance of CBD east of the Rift was reported by Rayner [10].

At the beginning, the disease was related to the fungus *C. coffeanum* described from Brazil by Noack [11] causing leaf spots on Arabica coffee. But the new disease in Kenya produced anthracnose-like symptoms on green berries. Rayner [10] called the pathogen *C. coffeanum* var. *virulans* to differentiate between leaf and berry symptoms. Morphological and pathogenicity research by several authors from the 1960s to 1990s finally resulted in the name *C. kahawae*, representing the Kiswahili word for coffee in the species name [12]. Prior to that time, the pathogen was called either CBD-strain [13] or *C. coffeanum* Noack sensu Hindorf [14,15]. Intensive investigations on the *Colletotrichum* population in coffee were carried out by Hindorf [16–18] and three distinct species occurring in association with CBD on coffee berries were described as (1) the CBD-causing species *C. coffeanum* growing with black colour on artificial Malt-Extract Agar, (2) *C. acutatum* with pink colour *in vitro* and (3) *C. gloeosporioides* producing symptoms only on ripe berries as the so-called late blight and a perfect stage of *Glomerella cingulata* [17].

From Kenya the disease spread to Angola in 1930, Zaire in 1937, Cameroon between 1955 and 1957, Uganda in 1959, Tanzania in 1964, Ethiopia in 1971 and Malawi in 1985 [19,20]. Until now the disease has been restricted to East, Central and South African coffee-growing regions. In Ethiopia the disease occurred much later than in neighbouring Kenya. After its first appearance in Sidamo and the first report by Mogk [21], the disease spread very quickly to nearly all growing coffee provinces until 1978 and caused remarkable losses. In the most restricted province of Hararghe the disease occurred only after 1985 and the coffee crop started being replaced by Chat, *Catha edulis* [22].

The pathogen can infect all organs of the host: flower buds, leaves, fruits and the maturing bark. Infection takes place either early during flower bud formation causing some losses in flowers or remains latent in the inflorescence until the berries start to expand in growth [23]. The outbreak of the disease with visible symptoms occurs during the expanding stage of berry development, producing sunken, black, anthracnose-like lesions on the green pulp. High moisture or pulp wetness favours the production of conidia in black acervuli appearing in concentric rings and exuding pink masses of one-celled, straight or slightly curved hyaline conidia. The conidia are splash-borne or distributed by insects, coffee pickers or other vectors, but never by wind due to a sticky constellation in the pink masses. In the absence of buds and berries the pathogen survives in the maturing bark of secondary branches. The pathogen never attacks mature coffee beans; it remains in the pulp. The losses occur during early infestation by destroying the beans or by preventing proper wet and dry processing since the pulp cannot be removed completely, causing so-called "stinkers" in the crop and reducing the quality. An intensive progress of the disease in the expanding stage of the berry development finally produces mummified berries with no economic value at all.

Information concerning the incidence of CBD in the Ethiopian forest coffee regions of Harenna, Bonga, Berhane-Kontir and Yayu is presented in Table 3 [24]. Assessments of the incidence (infected trees per locality) and severity (infestation of single trees) were scored visually. The CBD occurrence depended mostly on altitude ranges; higher sites were more frequently infected than lower sites due to more favourable climatic conditions for the pathogen (Fig. 4).

The pathogenicity of CBD isolates was not only tested on detached berries in the laboratory but also on seedlings in the greenhouse to investigate the diversity of coffee grown under natural conditions. Seedlings from seeds collected in Harenna, for instance, produced in the lower site 2 incidence rates of 23.3% and proved to be as similarly resistant/tolerant as resistant cultivars such as cv. 754 and 741. In contrast, on the higher site 3 of the same region only one tree with a lower intensity of 27.3% berries infected by the pathogen of CBD was found; all the other nine trees were highly susceptible.

Due to the fact that CBD was present in the surroundings of the Bonga and Yayu sites it was decided to carry out attached berry tests directly in the field, a well-documented method of testing CBD resistance. The pathogen isolates used for infection tests were collected from local field sites (Table 3). The infection tests on attached berries in the field sites of Bonga and Yayu produced a large diversity in susceptibility. Infection rates at Bonga varied in 2004 between 0% and 47.0% and in 2005 between 7.9% and 81.5%. Coffee trees were less susceptible at Bonga than at Yayu.

Coffee Wilt Disease (Plate 1C), *G. xylarioides* (*F. xylarioides*)

Coffee Wilt Disease (tracheomycosis) is a vascular disease caused by the fungal pathogen, *G. xylarioides* (*F. xylarioides*) and results in a total death of the infected coffee trees. The disease has been a serious problem to the production of Robusta coffee in DR Congo and Uganda since the 1990s killing hundreds of trees.

The first appearance on Arabica coffee in Ethiopia was reported in 1958 by Lejeune [25] and a diagnostic confirmation was provided by Kranz and Mogk [26]. The disease occurred first on some large scale state farms near Gera [27]. Detailed morphological studies and pathogenicity tests were carried out in a Ph.D. thesis by Adugna [28], who compared isolates from Arabica and Robusta coffee. In seedling tests it was proved that isolates from Arabica sources could only infect *C. arabica* and isolates from Robusta sources only infected *C. canephora* [28–31]. Therefore it was suggested, that the coffee wilt population should be classified into two formae speciales [32]: *G. xylarioides* f. sp. *abyssiniae* (*F. xylarioides* f. sp.

Table 3 Incidence and severity of CBD in the forest coffee areas of Ethiopia.

Locality	Sample site	Isolatecode	Altitude(m)	CBD incidence (%)	CBD severity (%)
I. Harenna	1	40	1683	30.0	8.5
	2	41	1715	40.0	15.0
	3	42	1656	1.0	1.3
	4	43	1674	50.0	13.0
	5		1532	0	0
	6	–	1451	0	0
	7	–	1420	0	0
Mean				18.6	5.4
SD				21.2	6.6
II. Bonga	1	50	1893	60.0	21.0
	2	51	1872	30.0	17.5
	3	52	1845	20.0	12.5
	4	53	1775	40.0	15.0
	5	54	1568	50.0	19.0
	6	55	1663	40.0	22.5
Mean				40.0	17.9
SD				14.1	3.7
III. Berhane-Kontir	1	60	1711	10.0	6.5
	2	61	1707	20.0	3.4
	3	–	1185	0	0
	4	–	1078	0	0
	5	–	1053	0	0
Mean				6.0	2.0
SD				8.9	2.9
IV. Yayu	1	70	1782	30.0	6.3
	2	71	1721	30.0	4.5
	3	–	1495	0	0
	4	–	1475	0	0
	5	72	1469	40.0	4.2
	6	73	1404	30.0	5.0
	7	74	1493	30.0	4.5
	8	75	1675	50.0	7.8
Mean				26.3	4.0
SD				17.7	2.8

abyssiniae) from *C. arabica* (Arabica) and *G. xylarioides* f. sp. *canephorae* (*F. xylarioides* f. sp. *canephorae*) from *C. canephora* (Robusta).

The pathogen exists on coffee trees in two developing stages: *Gibberella* as the sexual or perfect stage producing wind-borne ascospores and *Fusarium* as the asexual or imperfect stage with splash-borne conidia. Infection mostly takes place at the imperfect stage penetrating through wounds into the base of the stem. The fungus blocks the water supply in the vascular system and causes a typical brown discolouration. In the field, black to violet perithecia of the perfect stage are formed on or beneath the bark at the base of the stem. For the first time, Adugna et al. [31] produced perithecia of the perfect stage *in vitro,* when mating different isolates. The role of ascospores for distribution of the disease and in the infection process is not yet verified and needs to be investigated more precisely.

During the period of assessments of the disease in 2004–2006, CWD was detected in all the indigenous coffee field sites. The lowest percentage of infected trees was found in Berhane-Kontir, the highest in Yayu (Table 4). Seedling inoculation results showed that there existed significant differences among the tested accessions, and most of the coffee accessions collected from Harenna appeared to be highly resistant to CWD with infection rates between 0% and 4.0%. Some of the Bonga accessions had infection rates of 60–97%, Berhane-Kontir of 78–98% and Yayu of 56–98%. Seedlings of coffee accessions possessing moderate to high resistance to the CWD pathogen were grown, re-inoculated with the same fungus isolate and transferred to greenhouse and field sites for further observation.

Breeding for resistance to CLR and CBD in Kenya

Kenya is predominantly an Arabica coffee-producing country. Coffee was introduced into Kenya by missionaries at the beginning of the 20th century. The first plantations were established at Bura in the low lying coastal region of the country, but due to unfavourable climatic conditions, coffee growing was relocated to higher altitudes at Kibwezi and Kikuyu near the capital city of Nairobi. The first variety to be introduced and grown commercially was French Mission Coffee. Historically, cultivated Arabica coffee is derived from Bourbon and Typica types. In the early years of coffee cultivation, the breed-

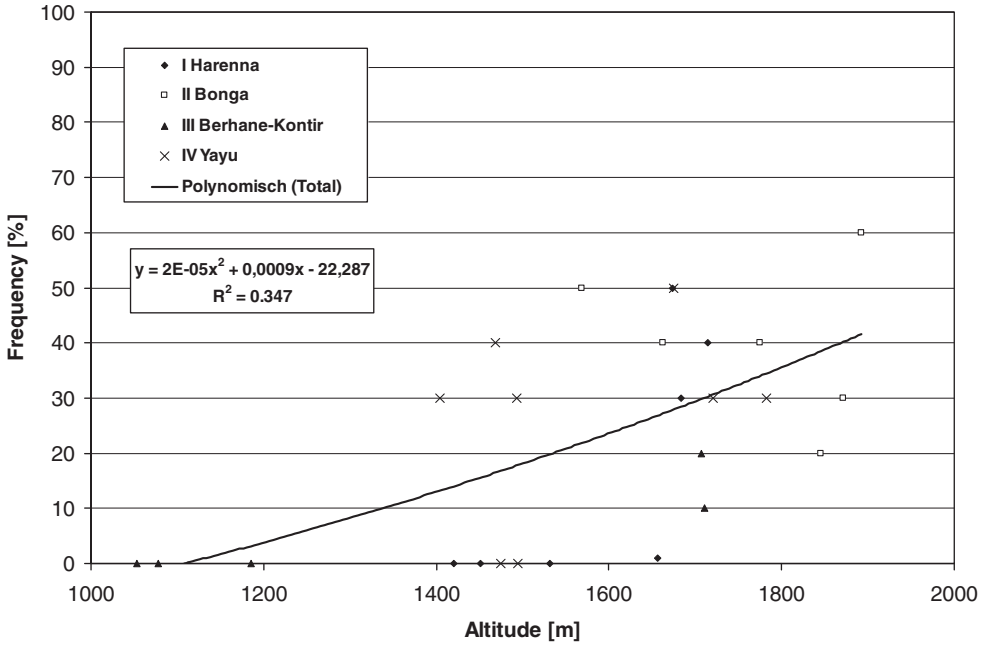


Fig. 4 Incidence of CBD in the forest coffee areas of Ethiopia [24].

ing objectives of most producing countries were to select varieties combining high yield, fine beverage quality and adaptation to local growing conditions. The breeding strategy was mainly by individual tree selections, giving rise to cultivars SL 28, SL 34 and K 7, which are still grown commercially today. Existing plantations of French Mission and Blue Mountain coffee varieties are the original accessions planted in Kenya before the selection process commenced.

C. arabica var. SL 28

The SL 28 cultivar was selected at the former Scott Laboratories (now the National Agricultural Laboratories, NARL situated at Kabete, Nairobi) on a single tree basis from the Tanganyika drought resistant variety selected in Northern Tanzania in 1931. The prefix "SL" is an acronym for Scott Laboratories, where the variety was selected. The name is completed by a serial number "28" for the selection. The variety is suited for medium to high altitude coffee-growing zones. It has predominantly green shoot tips, but occasionally bronze types can be observed. The angle of insertion of primaries is predominantly semi-erect, but tends to become decumbent or pendant after successive crop-bearing seasons. It has bold beans with particularly fine liquor and is susceptible to CBD, CLR and Bacterial Blight of Coffee (BBC), (*Pseudomonas syringae* pv. *garcae*).

C. arabica var. SL 34

SL 34 cultivar was also selected at the former Scott Laboratories from French Mission Coffee. The cultivar is adapted to high altitude areas with good rainfall. It is mainly characterized by dark bronze shoot-tipped plants with a few green-tipped strains. The laterals have a semi-erect habit, which tends to become decumbent or drooping on older primaries. The cultivar produces high yields of fine quality coffee, but is susceptible to CBD, CLR and BBC.

C. arabica var. K 7

K 7 cultivar was selected at Lengetet Estate in Muhoroni on the Lake Victoria basin from the French Mission Coffee. It is distinguished by its spreading habit on young laterals, although older primaries tend to be decumbent or drooping. The cultivar has characteristic medium to narrow leaves with young shoot tips that are an intermediate bronze in colour and shows resistance to some races of CLR, as well as partial resistance to CBD. It is suited to lower altitudes, where CLR is prevalent. The bean and liquor qualities are good.

Breeding objectives and selection methods

Although the above commercial varieties to a large extent met the original breeding objectives of combining high yield with good beverage quality and adaptation to the prevailing coffee-growing conditions, new challenges emerged that were hitherto not addressed in the selection process. Key among the challenges was CLR and CBD epidemics. Arabica coffee is also known to be genetically very narrowly based due its autogamous nature [33].

Breeding for resistance to CLR took into consideration the worldwide distribution of the disease and the multiple races of the pathogen. In 1955, the governments of the United States of America (USA) and Portugal established the Coffee Rust Research Centre (CIFC) in Oeiras, Portugal to coordinate CLR research without the risk of spreading new rust races to producing countries. Resistance to CLR is inferred from Flor's Gene-for-Gene concept, which states that for every major gene-conditioning resistance in the plant, there is a corresponding gene-conditioning virulence in the pathogen [34]. The resistance genes in the host are designated "S_H" genes while the virulence genes in the pathogen are designated "v". Resistance genes S_H 1–9 have been characterized and virulence genes v 1–9 have been inferred. In a collaborative effort be-

Table 4 Incidence of CWD in 2005 in the rainforest areas of Ethiopia.

Locality	Sample site[a]	Altitude (m)	CWD incidence (%)
I. Harenna	1	1683	0.0
	2	1715	6.0
	3	1516	12.0
	4	1531	10.0
	5	1519	8.0
	6	1476	16.0
	7	1298	16.0
Mean			9.7
SD			5.7
II. Bonga	1	1780	6.0
	2	1775	0
	3	1568	8.0
	4	1660	10.0
	5	1525	8.0
Mean			6.4
SD			3.5
III. Berhane-Kontir	1	1707	2.0
	2	1180	6.0
	3	1080	0
	4	1070	4.0
	5	1053	0
Mean			2.4
SD			2.6
IV. Yayu	1	1477	16.0
	2	1475	20.0
	3	1404	30.0
	4	1471	14.0
	5	1435	18.0
	6	1446	0
	7	1493	20.0
Mean			16.9
SD			9.0

[a] Number of samples: 30–50 trees/site.

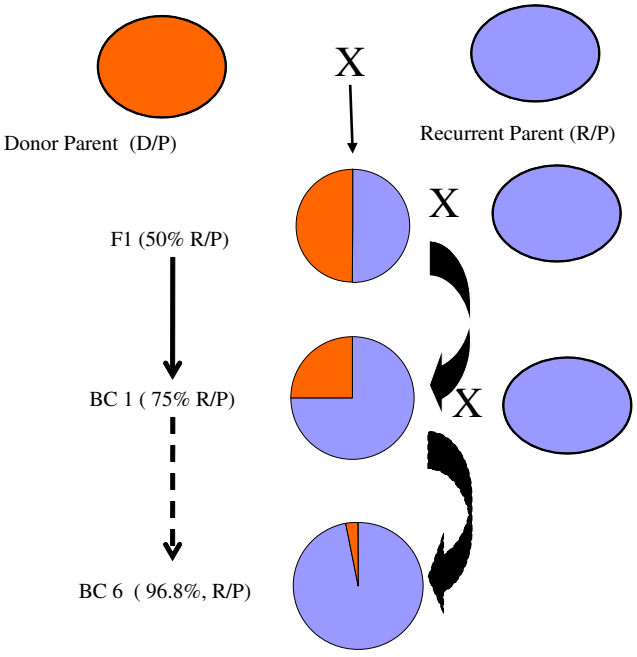


Fig. 5 Schematic presentation of the backcross breeding method.

tween CIFC and Arabica coffee-producing countries around the world, several varieties resistant to rust were developed. The most notable variety that was introduced in most countries was the Colombian Catimor, combining CLR and CBD resistance and compact growth.

In subsequent years, management of CLR and CBD became the main subject of research and novel control strategies combining chemical and cultural practices were developed to manage the two diseases. Despite intensive fungicide sprays, disease epidemics, particularly CBD, still contributed to significant economic losses, especially during prolonged cool and wet weather conditions. Analysis of coffee production costs further revealed that chemical control of CBD alone contributed up to 30% of the total [35]. It was further revealed that the continuous use of some fungicides, particularly Benzimidazole compounds was found to induce the emergence of fungicide-tolerant strains [36–40]. The fungicide-tolerant strains continued to persist in the pathogen population, even after the fungicides were withdrawn immediately after detecting the phenomenon [39].

Arising from these challenges, the breeding objective was expanded to include the search for and the deployment of resistance genes into existing commercial varieties that already had good yield, beverage quality and adaptability to coffee-growing conditions, using the backcross breeding method (Fig. 5). In Kenya, the breeding programme was initiated in 1971 as a bilateral partnership between the Kenya Government and the Netherlands Government. Realizing that the commercial cultivars grown in Kenya were mostly susceptible and that there was very little variability within the Arabica coffee germplasm in Kenya, an aggressive campaign to introduce accessions and landraces from other coffee-growing countries in Latin and Central America and particularly from the centre of origin of Arabica coffee in the southwest highlands of Ethiopia, was launched. The resulting genetic pool, comprising of the world coffee germplasm collection and the introductions of the 1964 FAO coffee mission to Ethiopia, provided the source of genetic variation from which to select for resistant genotypes [41]. Inheritance studies using 11 Arabica coffee varieties varying in CBD resistance revealed three major genes on separate loci [42]. The highly resistant variety, Rume Sudan originating from the Boma Plateau in southern Sudan, carries the dominant R- and the recessive k-gene. The R-locus has multiple alleles with R_1R_1 in Rume Sudan and R2R2 in Pretoria, which also carries the recessive k-gene. The moderately resistant variety K 7 carries the recessive k-gene. Clone 1349/269 of the variety Hibrido de Timor and its hybrid derivative Catimor carries one gene for CBD resistance on the T-locus with intermediate gene action.

A gene deployment strategy that would combine two or more resistance genes in the same plant and create variability through gene recombination in segregating populations arising from single, double, three way and multiple crosses was initiated. The resulting crosses were backcrossed to the susceptible commercial varieties to restore good yield, fine beverage qual-

ity and adaptability to local growing conditions while selecting for resistance in the resultant progeny as inherited from resistant donor parents (Fig. 5).

The breeding programme got a boost when the Catimor variety was introduced from Colombia. It was found to be resistant to CBD on the T-locus and to all the races of the CLR pathogen found in Kenya. The variety was also compact in growth, which presented an opportunity for high density planting. However, it could not be released as a commercial variety in Kenya, because the genetic base for CBD resistance was narrow (one gene) and the beverage quality required to be improved to the standard of SL 28, SL 34 and K 7. A strategy was adopted to use the Catimor variety as mother parent and the progeny of the backcross breeding programme cited above as the male parent in a hybrid seed production scheme. A variety combining the attributes of the Catimor variety and the backcross progeny was released in 1985 and named ''Ruiru 11''.

C. arabica var. Ruiru 11

The variety name has the prefix ''Ruiru'' referring to the location of the Kenyan Coffee Research Station where the variety was developed. The name is completed by an additional two code numbers, ''11''. The first code number denotes the sequence of release, in this case the first release, and the second number defines the type of variety as a one-way cross between two designated parent populations. The variety is not only resistant to CBD and CLR but is also compact in growth, allowing farmers to intensify the production per unit of land, especially in high potential areas, where the human population is high and coffee is in competition with other crops and farm enterprises required for food security and income. Ruiru 11 is planted at a density of 2500–3300 trees/ha compared to 1300 trees/ha for traditional varieties. This translates into a higher production per unit area of land. The variety comes into production earlier, hence earlier realization of benefits for farmers. The development of Ruiru 11 also took into consideration the importance of quality as a major marketing parameter. Since the quality of the traditional varieties was already popular among consumers of Kenyan coffee, Ruiru 11 was developed with quality attributes similar to the traditional varieties, SL 28, SL 34 and K 7.

Despite the successful performance of the Ruiru 11 variety, the major drawback has been the availability of adequate seeds to meet the high demand of growers both locally and in the region. As a hybrid variety, seed multiplication involves artificial cross pollination between the male and female parents. Noting that there has been no male sterility documented in coffee, artificial cross pollination requires manual emasculation of the female plants and pollination by the male plants. This is a labour intensive process that has continued to limit the amount of seeds that can be produced. Following the large scale cultivation of Ruiru 11 over several years, it has also been necessary to study the variation in the CBD pathogen. There has been no evidence of breakdown of resistance but differences in the aggressiveness of isolates are sometimes prominent [43].

CWD has not been reported in Kenya despite its close proximity to Uganda where the disease has ravaged Robusta plantations, because Kenya is predominantly an Arabica coffee-producing country. Ethiopia, which shares its southern border with Kenya, is the only country, where CWD has been detected on Arabica coffee, but it is believed that the arid Northern province of Kenya provides a buffer zone, hindering the spread of the disease into Kenya's coffee plantations. Breeding for resistance to CWD has therefore gained prominence in Uganda and Ethiopia, where the main focus is selection within the local landraces.

Recent progress in the variety improvement and development of a true breeding resistant variety

A breeding approach to develop a true breeding variety is currently in progress in Kenya. The variety has been entered into a pre-release adaptation trial. It was developed from individual tree selections of backcross progeny involving SL 4, N 39, Hibrido de Timor and Rume Sudan as the donor varieties and cvs. SL 28, SL 34 and K 7 as the recurrent parents. In this method, the best individuals within the best families were selected solely on the basis of their phenotypic values (within the family selection method). The strategy involved simultaneous selection for the important traits, but independent rejection of all the individuals that failed to meet the required standard for any one of the traits under improvement (independent culling level). The performance of cultivar Ruiru 11 was used as a standard check for discriminating against inferior lines when selecting for resistance to CBD and CLR, yield and quality. The variety SL 28 was also used as a standard when selecting for yield and quality.

The variety is a composite of five crosses (cross 8, cross 22, cross 23, cross 27 and cross 30) that are tall in stature, the distinctive features being true breeding, resistance to CBD and CLR. It is a high-yielding variety with good bean and liquor quality that is comparable to Ruiru 11 and SL 28, suited for all coffee agro-ecological zones in Kenya and has a conical shape with a horizontal but occasionally erect branching habit, which tends to become semi-drooping or drooping after successive crop-bearing seasons. The young leaves have medium anthocyanin colouration giving a bronze colour, occasionally absent or weak, giving a green-bronze colouration. Yield data indicate that the crosses are better than or comparable to the standard check varieties, Ruiru 11 and SL 28 (Table 5). Disease assessment data revealed that CBD infections were significantly higher in the susceptible SL 28 than in the treatments and resistant Ruiru 11 control (Table 5). CLR infection showed clear variations between the susceptible SL 28 on the one hand and the resistant crosses and Ruiru 11 control on the other. It is important to note that resistance among the crosses was not significantly different from the resistant Ruiru 11.

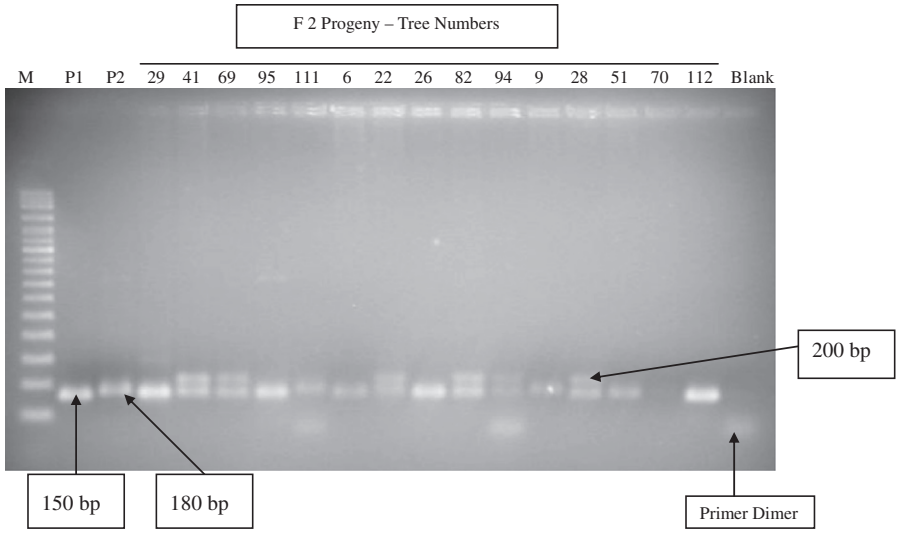
Molecular approaches to coffee breeding

Efficient and reliable disease screening methods are required for a successful variety development programme. Molecular markers linked to resistance provide the potential to screen for resistance in a large population of plants at any stage of plant development. Where several genes confer resistance, markers have the advantage over morphological assessments, because plants carrying multiple resistance (broad-based resistance) can easily be differentiated from those carrying a single gene (narrow-based resistance). Attempts have been

Table 5 Mean yield performance and disease score of the five test genotypes and control varieties (Ruiru 11 and SL 28) at Tatu Estate in Ruiru/Kenya.

Treatment	Mean yield of clean coffee (g/tree)	Disease score			
		28.6.2007		26.7.2007	17.9.2007
		Mean CBD	Mean CLR	Mean CLR	Mean CLR
Cross 08	1718.66BC	0.12A	0.1833AB	0.2333AB	0.2667A
Cross 22	1045.93D	0.3267A	0.45C	0.4667B	0.4167A
Cross 23	1966.36AB	0.0967A	0A	0.15AB	0.1333A
Cross 27	1292.80CD	0.1433A	0.35BC	0.4333B	0.3833A
Cross 30	2539.03A	0.0333A	0.3BC	0A	0A
Ruiru 11	1877.40BC	0.1133A	0.1333AB	0.2167AB	0.15A
SL 28		1.07B	1.0167D	1.4333C	2.1667B

Note: Means followed by a common letter(s) are not significantly different according to Duncan's Multiple Range Test ($P = 0.05$).



M = 100 bp ladder, P1 =SL 28, P2 = Rume Sudan.

Fig. 6 SSR polymorphism using primer M2.

made to screen for DNA markers linked to CBD resistance in Catimor and Rume Sudan coffee varieties [44,45]. For instance, DNA was extracted from an F2-mapping population derived from Rume Sudan × SL 28 using the method of Diniz et al. [46]. The DNA was subjected to microsatellite analysis using primer M 24 that had forward and reverse sequences 5′GGCTCG AGATATCTGTTTAG3′ and 5′TTTAATG-GGCATAGGG TCC3′, respectively, and repeat motif as (CA)15(CG)4CA [47]. The primer detected polymorphism at three different levels (Fig. 6). The susceptible parent, SL 28, amplified a fragment of 150 bp size, while the resistant parent, Rume Sudan, amplified a fragment of about 180 bp. These fragments were also evident in some F2 progeny. The third category of fragments appeared in pairs and was mainly observed in the F2 plants. This category is believed to comprise the heterozygotes. Omondi et al. [45] concluded that the observed SSR polymorphism is consistent with major gene inheritance. The resistant Rume Sudan variety is known to carry a dominant gene for CBD resistance on the R-locus [42]. More investigations are still necessary to establish with precision the trait that co-segregates with the observed DNA bands so as to conclude that the bands that represent markers for a specific target trait. Efforts have now been directed to determine the genotypes of individual plants constituting of the mapping population using the hypocotyls inoculation test. The potential use of the bands as markers for selection will depend on their potential to co-segregate with resistance/susceptibility to CBD.

Conclusions

Ethiopia is the centre of origin of *C. arabica* and there exists an immense opportunity to develop and use resistant varieties to manage diseases. The existence of a tremendous diversity of different characteristics was observed in Arabica coffee

[4,47,48,51]. This observation was recently ascertained by molecular analysis [49,50]. Various investigations demonstrated the presence of resistance to CLR, CBD and CWD in Arabica coffee collected from Ethiopia [4,7,24,50].

Tremendous achievements have been realized by breeding coffee varieties resistant to fungal diseases. Resistant varieties have the potential to reduce the cost of production and offer environmentally better disease management approaches. Novel methods of selection that can reduce the time taken for variety development are being explored through molecular marker approaches.

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