- Subscribe / Renew

FOR CONTRIBUTORS

- OnlineOpen
- Author Guidelines
- Submit an Article

ABOUT THIS JOURNAL

- Society Information
- News
- Overview
- Editorial Board
- Permissions
- Advertise
- Contact

SPECIAL FEATURES

- Special Issue
- Wiley Job Network
- Virtual Issue - The phenomenon of resistance to fungicides
- Virtual Issue - Emerging Diseases of Trees and their Management

Plant Pathology
An International Journal edited by the British Society for Plant Pathology

Identification of molecular markers linked to a gene conferring resistance to coffee berry disease (*Colletotrichum kahawae*) in C*offea arabica*

1. E. K. Gichuru[1],
2. C. O. Agwanda[1,†],
3. M. C. Combes[2],
4. E. W. Mutitu[3],
5. E. C. K. Ngugi[3],
6. B. Bertrand[4],
7. P. Lashermes[2,*]

Article first published online: 27 MAR 2008
DOI: 10.1111/j.1365-3059.2008.01846.x

Issue

Plant Pathology

Volume 57, Issue 6, pages 1117–1124, December 2008

- †
  Present address: Coffee Research Network (CORNET), CAB International – Africa Regional Centre, P. O. Box 633-00621, Nairobi, Kenya.

Keywords:

- Coffee berry disease;
- *Colletotrichum kahawae*;
- *Coffea arabica*;
- introgression;
- resistance;
- marker assisted selection

Abstract

Coffee berry disease (CBD) caused by *Colletotrichum kahawae* is a major constraint to Arabica coffee (*Coffea arabica*) production in Africa. One source of resistance to the disease is a natural interspecific hybrid between *C. arabica* and *C. canephora* and its derivatives. This study is aimed at deciphering the genetic basis of the host resistance and identification of molecular markers associated with it. CBD is a mature stage disease and in the absence of a mature mapping population, early detection of disease reaction phenotypes of mapping individuals is required. Two F_2 populations from crosses of cv. Catimor (resistant) and cv. SL28 (susceptible) were screened for resistance by a two step procedure. First, half of each population was screened 6 weeks after germination by inoculating hypocotyls with the pathogen. The surviving seedlings (G1) were considered to be resistant and were raised in a nursery together with the other unscreened halves (G2). Secondly, after one year, all the seedlings (G1 + G2) were screened by inoculation. Analysis of 57 microsatellites and 31 AFLP markers in 56 and 95 seedlings from G1 and G2, respectively, were performed. Eight AFLP and two microsatellites markers linked tightly to the resistant phenotype were identified and mapped to one unique chromosomal fragment introgressed from *C. canephora*. The gene conferring the resistance was localized within an 11 cM segment. It is concluded that the locus carries a major resistance gene designated *Ck*-1, which is likely to be synonymous to the *T* gene described in previous studies.

Introduction

Coffee is an important export commodity in many countries in Latin America, Africa and Asia. It provides a livelihood for over 120 million people worldwide (Osorio, 2002). Arabica coffee (*Coffea arabica*) accounts for about 63% of the total world coffee production, the rest being mainly Robusta coffee (*C. canephora*). Arabica coffee is preferred for its superior beverage quality, but crop production is often constrained by diseases. Chemical control alone may account for more than 30% of field costs (Nyoro & Sprey, 1986), which makes coffee growing unprofitable in years of low prices on the world market, for the smallholder farmers in particular. Coffee berry disease (CBD) is an anthracnose caused by *Colletotrichum kahawae* that may cause severe crop losses on Arabica coffee in Africa whenever climatic conditions are favourable to the pathogen (Griffiths *et al*., 1971; Van der Graaff, 1978; Masaba & Waller, 1992). Development of disease resistant crop cultivars is one major objective of many breeding programmes, since resistant cultivars reduce the costs of using pesticides and are safe to humans and environment. Many

commercial cultivars of *C. arabica* are susceptible to diseases and introduction of resistance genes involves crossing with donor varieties, followed by backcrossing to restore desirable traits, especially yields and quality. In so doing, the use of molecular marker assisted selection (MAS) would be particularly useful (Rieseberg *et al*., 2000).

Coffea arabica is the only tetraploid species ($2n = 4x = 44$) of the genus *Coffea* and originated from the union of the diploid genomes of *C. canephora* and *C. eugenioides* (Raina *et al*., 1998; Lashermes *et al*., 1999). Despite many morphological differences, *C. arabica* exhibits very low diversity at DNA level, which is attributed to its allotetraploid origin, selfing reproductive nature and recent speciation (Lashermes *et al*., 1999). Introduction of desirable agronomic traits from other *Coffea* species can be achieved by use of natural or artificial inter-specific hybrids (Lashermes *et al*., 2000a). For example, the plant called Hibrido de Timor is a spontaneous inter-specific cross between *C. arabica* and *C. canephora* that originated on the island of Timor (Bettencourt, 1973). Progenies of Hibrido de Timor have been used worldwide as a source of resistance to various diseases including CBD, coffee leaf rust and nematodes. Progenies of Hibrido de Timor and advanced inbred lines of its cross to *C. arabica* cv. Caturra, (referred to as cv. Catimor), have been screened for CBD and leaf rust resistance and are used as donor parents in Kenya. Based on inheritance studies, Van der Vossen & Walyaro (1980) proposed the existence of one locus (*T*) for CBD resistance in Hibrido de Timor.

The development of DNA markers linked to CBD-resistance genes would considerably increase the efficiency of breeding programmes by allowing for selection at an early stage in a large number of breeding lines, and gene stacking to increase chances of high levels of durable resistance (Lashermes *et al*., 2000b). In addition, it would allow pre-emptive breeding in countries where CBD is not yet present (Latin America, Asia), but climatic conditions are often favourable. Agwanda *et al*. (1997) identified randomly amplified polymorphic DNA (RAPD) markers of CBD resistance derived from Hibrido de Timor but their use is limited by low reproducibility. Two DNA marker systems of particular interest are AFLPs (amplified fragment length polymorphisms) and microsatellites (i.e. simple sequence repeats, SSR) which differentially present the advantages of wide genome coverage, reproducibility, large number of data points developed in one reaction and high information content (Vos *et al*., 1995; Rafalski *et al*., 1996; Li *et al*., 2002).

To map a trait, the phenotype of the individuals of the mapping population has to be characterized. CBD is a mature stage disease and in the absence of a mature mapping population, a method for early detection of disease reaction phenotypes is required. The hypocotyls-inoculation method (Van der Vossen *et al*., 1976), widely applied for early detection of CBD resistance, is of rather limited use for molecular mapping, as CBD-susceptible seedlings are usually completely destroyed by the pathogen before DNA can be extracted from healthy plant tissue. Another inoculation test on shoot-tips of 10-month-old seedlings, described by the same authors, allows enough unaffected leaf tissue for DNA extraction on susceptible plants. However, the sensitivity and repeatability of the shoot-tip test is much lower compared to the hypocotyl inoculation test, due to higher environmental variation and consequently more escapes. Another difficulty is the occurrence of some uninfected seedlings in susceptible genotypes and fully susceptible reactions in resistant genotypes, aspects which may reduce efficiency of marker identification. While the occurrence of unaffected seedlings in otherwise susceptible populations may be attributed to escapes, susceptible seedlings in CBD-resistant-populations are probably abnormally weak aneuploids which are known to occur at frequencies of 1–3% in populations of the allotetraploid *C. arabica* (Van der Vossen & Walyaro, 1980). Both phenomena do, of course, complicate the gene mapping exercise.

The objectives of this study were: (i) to identify a suitable method for early screening of a mapping population for CBD resistance; (ii) to identify and map DNA markers linked to CBD-resistance gene(s) introgressed into *C. arabica* from *C. canephora* through the Hibrido de Timor; and (iii) to evaluate the prevalence of identified markers in diverse populations of Hibrido de Timor.

Materials and methods

Plant materials

Two mature F_1 coffee trees resulting from crosses between the pure lines cv. SL28 (as female) and either the cv. Catimor 127 or the cv. Catimor 88 were selfed in breeding fields at the Coffee Research Foundation (CRF), Ruiru Kenya, to obtain two F_2 populations (Pop 1 and Pop 2, respectively). Seeds of the susceptible cv. Caturra were also harvested from the same field for use as controls to confirm successful infection during inoculation tests. Five weeks after germination, each F_2 and cv. Caturra seed lot was divided into halves. One half of each seed lot (G1) were retained in the laboratory for CBD resistance screening at the

hypocotyl stage while the other half (G2) were potted into polythene bags, 10 × 20 cm, and transferred to a nursery for screening later as young seedlings.

Screening for CBD resistance

Experiment 1: Inoculation of seedling hypocotyls (G1 plants)

The seedlings targeted for screening at the hypocotyl stage (G1) were inoculated by the method of Van der Vossen *et al*. (1976) using a pathogenic single-spore isolate (KW33) from a collection of *C. kahawae* isolates maintained by the Plant Pathology Department of CRF. The G1 seedlings were planted in two lots as replicates. Initially, the isolate was inoculated onto detached green immature coffee berries of cv. SL28 and re-isolated to ensure optimal pathogenicity. The plants were scored using a 1 to 12 scale in increasing order of susceptibility (Van der Vossen *et al*., 1976). Seedlings within Classes 1 to 4 from the two F_2 populations were categorized as resistant, and transferred to the nursery as resistant sub-populations (G1) for cross checking in later inoculation and molecular studies.

Experiment 2: Inoculation of young seedlings

The resistant seedlings (G1, two replicates combined), the G2 seedlings from the two F_2 populations, plus seedlings of cv. Caturra, were maintained in the nursery without fungicide application for one year before being inoculated. Seedlings were arranged in boxes carrying 32 potted seedlings, for ease of handling. In each box, two seedlings of the susceptible cv. Caturra were randomly placed among the F_2 seedlings as susceptible controls. A box containing only seedlings of cv. Caturra was included as an extra control. The seedlings were transferred to the laboratory and inoculated with the same isolate used for inoculation of hypocotyls (2×10^6 conidia mL^{-1}) by spraying the top part of the seedlings (up to the third node). For infection, the seedlings were covered with dark polythene sheets and humidified for 48 h at room temperature (22–24°C), and then transferred into a cooled incubation room (18 ± 2°C) for 3 weeks before being transferred back to the nursery. After 2 weeks in the nursery, infection was assessed based on all aspects of pathogenesis observed during the entire screening process. A five class scoring scale was used to categorize the seedlings into different phenotypes of resistance to infection by *C. kahawae*. In the scale, class 0 was symptomless, classes 1 and 2 had limited infections on the upper young parts that mostly resulted in scabs; class 3 exhibited larger black lesions sometimes mixed with scabs, girdling of nodes and killing of topmost internodes; class 4 had rapidly expanding active lesions killing large parts of the seedlings or whole seedling.

Identification of candidate markers for CBD resistance

One week before the inoculation, leaves were sampled from all the F_2 seedlings, two accessions of the susceptible parent (cv. SL28) and the resistant parents (cv. Catimor 88 and cv. Catimor 127). The leaves were then lyophilized and stored in a cold room at 4°C until required for DNA extraction. Genomic DNA was extracted from 50–100 mg of the lyophilized leaves by the method of Diniz *et al*. (2005). To identify candidate markers for CBD resistance, the four parental accessions and 27 F_2 plants of G1 (screened as resistant by the hypocotyls-inoculation method) from Pop 2 were analysed with 31 AFLP primer combinations and 57 microsatellite primer pairs. AFLP analysis was done following the protocol of Vos *et al*. (1995) as adopted by Lashermes *et al*. (2000a) for coffee. The 31 *Eco*RI and *Mse*I primer combinations were chosen to maximise polymorphism in relation to introgressed *C. canephora* chromosomal fragments (Lashermes *et al*., 2000a). AFLP bands were named by the three selective nucleotides of the primer combinations (*Eco*RI followed by *Mse*I) and a letter in increasing alphabetical order from the largest band. Microsatellites were analysed by the radioactive method described by Combes *et al*. (2000). Banding patterns were visualized either on Kodak Biomax X-ray films or on Amersham Phosphor storage screen and scanned with a Typhoon scanner (9700 series, Amersham Biosciences) to obtain digital images.

Identification of CBD resistance markers

Twenty nine G1 plants from Pop 1 and 95 G2 plants from Pop 2 were analysed for further confirmation and mapping of the candidate markers. The 95 plants covered all the phenotypes of reaction to CBD as identified by inoculation at the young seedling stage. The segregation of markers associated with CBD resistance in the F_2 populations were tested for goodness-of-fit to the expected Mendelian ratios by chi-squared (χ^2) analysis. Mapping was done based on the G2 plants using MapMaker Version 3·0b (Lander *et al*., 1987) with an initial logarithm of odds (LOD) score of 5·0 to identify linked markers and then lowered to 3·0 for the actual mapping.

Survey of CBD resistance markers in field-evaluated Hibrido de Timor-derived lines

Five Hibrido de Timor-derived lines selected by the Coffee breeding unit of CRF in Kenya were used in this study. Four lines corresponded to CBD-field resistant material as evaluated by long term field observations (since 1992) while the fifth line showed high susceptibility to CBD in field conditions. Genomic DNA was extracted from lyophilized leaves from the mother plants and amplified with microsatellites linked to the resistance, i.e. Sat 207 (forward primer GAAGCCGTTTCAAGCC, reverse primer CAATCTC TTTCCGATGCTCT) and Sat 235 (forward primer TCGTTCTGTCATTAAATCGTCAA, reverse primer GCAAATCATGAAAATAGTTGGTG). During this phase, the repeatability of the microsatellite and spaced AFLP markers was tested by re-extraction and amplification of the G1 plants from the two populations that had already been established in the field for future studies.

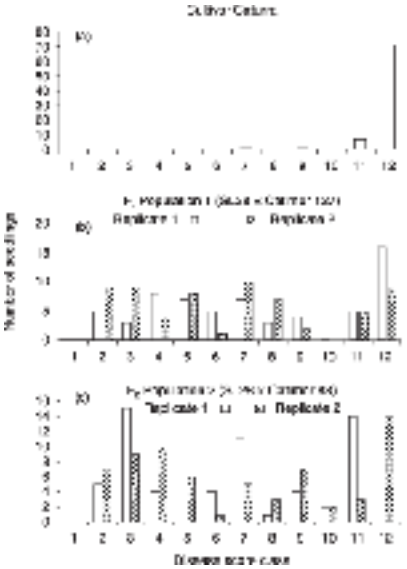
Results

Phenotypic screening of F_2 populations for CBD resistance

Experiment 1: screening of seedling hypocotyls

Two F_2 populations, Pop 1 and Pop 2, derived from resistant cvs Catimor 127 and Catimor 88, respectively, crossed with susceptible cv. SL28 were assessed for resistance in two replicate experiments by the hypocotyl inoculation method (Van der Vossen *et al*., 1976), using a pathogenic single spore isolate of *C. kahawae*. The success of infection was evaluated by comparison with severity on the susceptible control cv. Caturra. The majority (97·5%) of the 80 cv. Caturra seedlings were in the highly susceptible classes 11 and 12 (Fig. 1a) with a mean score of 11·8, and therefore infection was highly successful. However, the F_2 populations segregated into all the classes, except the very resistant class 1 (Fig. 1b,c). The distribution of seedlings within the infection classes was similar between replicates and populations, although Pop 1 appeared to be more evenly spread out than Pop 2. The mean infection grades of the first and second replicates of Pop 1 were 7·6 and 6·5, respectively and an overall mean of 7·1. In Pop 2, the mean infection grades were 7·3 and 6·9, respectively, for first and second replicates, with an overall average of 7·1. There were no significant differences in the distribution of the seedlings between replicates of the same population ($\chi^2 = 1{\cdot}05$; $P = 0{\cdot}300$ and $\chi^2 = 0{\cdot}85$; $P = 0{\cdot}336$ for Pop 1 and 2, respectively), nor between the two F_2 populations ($\chi^2 = 0{\cdot}21$; $P = 0{\cdot}646$). By comparing the infection results of the F_2 populations with those of the susceptible cultivar, seedlings in classes 11 and 12 were considered as susceptible and the rest of the seedlings were considered to express resistance. Using this criterion, the ratios of resistant to susceptible seedlings were 96:35 and 103:44 in Pop 1 and 2, respectively. These ratios fitted a 3:1 ratio for a major gene action ($\chi^2 = 0{\cdot}206$ $P = 0{\cdot}650$ and $\chi^2 = 1{\cdot}907$; $P = 0{\cdot}167$ for Pop 1 and 2, respectively). However, the spread of the reaction phenotypes suggested lack of strict dominance, presence of other modifying genes or gene by environment interaction. Despite the above categorization of susceptibility versus resistance, only seedlings within classes 1 to 4 were used in subsequent studies as resistant sub-populations (G1). Susceptible seedlings were eliminated by the infection before DNA could be extracted from them.

Figure 1. Seedling hypocotyl scores of duplicates of two F_2 populations of *Coffea arabica* after inoculation with *Colletotrichum kahawae*. Replicates 1 and 2 refer to first and second harvest of each population: (a) susceptible cv. Caturra, (b) Population 1 (cv. SL28 × Catimor line 127) and (c) Population 2 (cv. SL28 × Catimor line 88).



Experiment 2: screening of young seedlings

Two subsets of the two F_2 populations were evaluated for CBD resistance after one year by inoculation with the same isolate that was used during hypocotyl inoculation tests. These were G1 plants, earlier identified as resistant by inoculation at the hypocotyl stage, and G2 plants, which were not screened. Before inoculation, leaves were sampled from all seedlings to ensure availability of DNA for later studies. Symptoms of infection were scored visually 5 weeks after inoculation. The severity of infection varied from none to complete death of the seedlings. Most infections on the older part of the seedlings started at the nodes, especially where defoliation occurred, and the resultant girdling killed the upper parts even when lesions did not extend into the inter-node areas. Eight out of 11 small (non-vigorous) seedlings were rapidly killed within the first 3 weeks, which casts doubt on their reliability for phenotype identification, and were not subsequently categorized phenotypically. However, the plants which were not categorized into CBD-resistance phenotypes were included in molecular analysis of the integral F_2 population, to avoid segregation distortion of markers. A five class scoring system was developed, where 0 = no observable symptoms; 1 and 2 = limited infections on the upper young parts mostly resulting in scabs; 3 = larger black lesions sometimes mixed with scabs, girdling of nodes and killing of topmost internodes; and 4 = most susceptible, marked by rapidly expanding active lesions that caused death to large parts of the seedlings or complete seedling death, sometimes with pathogen sporulation. The distribution of the assessed seedlings into the various classes is presented in Table 1. Classes 0–2 were considered to be resistant and class 4 to be susceptible. Plants in class 3 could not be clearly categorized as resistant or susceptible due to a mixture of symptoms that were observed in pure line progenies of both the resistant and susceptible parents during preliminary testing of the methodology. This class also had many plants which were of low vigour, especially in girth, and were mainly girdled at the nodes thus killing the upper parts. The low vigour plants (small or thin) were more frequent in Pop 1 than in Pop 2. Five plants of cv. Caturra (9·8%) were placed into classes 0–2 and were interpreted as failed infection. Two of these plants had dormant shoot tips although the topmost leaves were dark green and fully expanded. Three plants (10·3%) from G1 of Pop 1 were classified as susceptible, although they were resistant in hypocotyl screening tests. The three plants were of low vigour and had thin stems relative to height. Based on these results, Pop 1 was considered to be more liable to misclassifications and Pop 2 was chosen for molecular analysis in identification and mapping of markers for CBD resistance. The G1 plants of Pop 1 were used as a resistant sub-population for cross-checking.

Table 1. Resistance reactions to inoculation by *Colletotrichum kahawae* of 1-year-old seedlings of F_2 populations of *Coffea arabica* cv. SL28 crossed with cv. Catimor 127 (Population 1) and cv. Catimor 88 (Population 2). The susceptible cv. Caturra was included as control. The observation was made on the fifth week after inoculation

Plant material		Seedlings assessed[a]	Resistant (Classes 0–2)	Intermediate (Class 3)	Susceptible (Class 4)
cv.Caturra		51	9·8 [b]	20·6	70·6
G1[c]	F_2 Population 1	29	68·9	20·8	10·3
	F_2 Population 2	35	77·1	22·9	0·0
G2[d]	F_2 Population 1	95	40	25·3	34·7
	F_2 Population 2	121	47·9	30·5	21·6

- [a] Seedlings were inoculated by spraying the top part of the plant with a solution of 2×10^6 conidia mL^{-1}.
- [b] Results are expressed as percentages of seedlings in each class.
- [c] G1 = resistant individuals that had survived hypocotyl inoculation.
- [d] G2 = seeds without prior selection.

Identification of candidate AFLP and microsatellite markers for CBD resistance

Thirty-one AFLP primer combinations were screened against three parental representatives comprising cv. SL28 (susceptible), cv. Catimor 88 and cv. Catimor 127 (resistant) and 27 resistant G1 plants from Pop 2. These primer combinations generated 1 to 9 polymorphic bands each, with a total of 96 polymorphic bands. Fifty-seven microsatellites (Combes *et al*., 2000; Mahé*et al*., 2007) were screened in the same accessions. In total, nine AFLP bands and three microsatellite alleles derived from the two cv. Catimor plants were present in over 90% of the F_2 plants resistant to CBD and were considered to be candidate markers for resistance.

Identification of markers linked to CBD resistance

The candidate markers for CBD resistance were analysed in 95 G2 plants of Pop 2 as shown in Fig. 2. Eight AFLP and two microsatellite markers had observed values that were consistent with the expected ratios of 3:1 and 1:2:1, respectively. The other two markers (one microsatellite and one AFLP marker) that were initially considered to be candidate markers had distorted segregation in the integral G2 plants ($\chi^2 = 14{\cdot}806$; $P = 0{\cdot}001$) in favour of the alleles derived from the two lines of cv. Catimor, and they perfectly co-segregated. They did not appear to be linked to CBD resistance and were not considered further. Linkage analysis was performed for the 10 markers associated with CBD resistance. All markers appeared to be tightly linked to each other (LOD score > 5) and mapped onto one unique fragment (Fig. 3). One AFLP band (ACC-CAA-f), which had not been selected as a potential marker, co-segregated to a large extent with another band that was amplified by the same primer combination (i.e. ACC-CAA-e), and was therefore also mapped onto the fragment.

Figure 2. An example of the pattern of Sat 235 in F_2 *Coffea arabica* plants resistant and susceptible to infection by *Colletotrichum kahawae*.

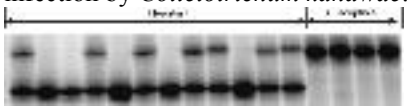
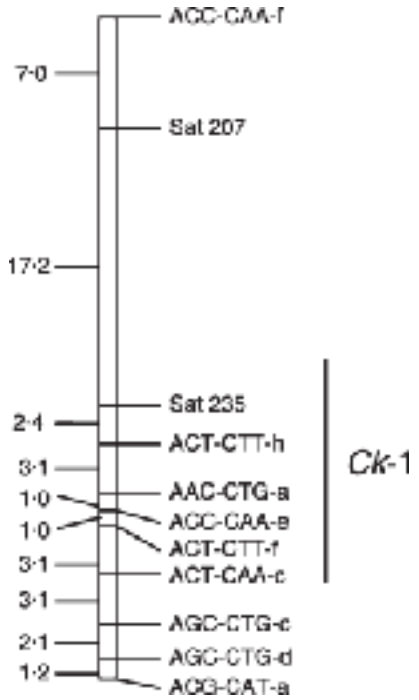


Figure 3. Genetic linkage map of markers found to be associated with coffee berry disease (CBD) resistance based on the *Coffea arabica* F_2 (SL28 × Catimor 88) population. The values on the left are the distances between the markers in cM. The segment carrying the C*k*-1 locus of resistance to CBD is delimited based on the evaluation of G1 plants (Table 2).



Further confirmation of the markers for CBD was done by analysis in G1 plants (resistant) from the second F_2 population (Pop 1). These exhibited a similar distribution with more than 90% presence of markers. It was also observed that all the resistant G1 plants analysed for molecular markers (29 plants from Pop 1 and 27 plants from Pop 2) had the markers of the mapped fragment from Sat 235 to ACT-CAA-c (Table 2). It was consequently concluded that the resistance gene is located between Sat 207 and AGC-CTG-c or close to either of these markers, a distance of about 11 cM (Fig. 3). The designation *Ck*-1 was used to refer to the locus for resistance to *C. kahawae*. No recombination was observed between the markers Sat 235 and ACT-CAA-c in G1 plants, and therefore locality of the gene could not be further refined.

Table 2. Proportion of *Coffea arabica* plants exhibiting the candidate markers. Only plants from the two populations tested (cv. SL28 × cv. Catimor 88 and cv. SL28 × cv. Catimor 127) that presented a high level of resistance to coffee berry disease in the seedling hypocotyl test were analyzed

Markers	**Proportion of plants exhibiting the markers**		
	Population 1 (29 seedlings)	**Population 2 (27 seedlings)**	**Population 1 and 2 (56)**
Sat 207	96·5	92·6	94·6
Sat 235	100	100	100
ACT-CTT-h	100	100	100
AAC-CTG-a	100	100	100
ACC-CAA-e	100	100	100
ACT-CTT-f	100	100	100
ACT-CAA-c	100	100	100
AGC-CTG-c	96·5	100	98·2

Table 2. Proportion of *Coffea arabica* plants exhibiting the candidate markers. Only plants from the two populations tested (cv. SL28 × cv. Catimor 88 and cv. SL28 × cv. Catimor 127) that presented a high level of resistance to coffee berry disease in the seedling hypocotyl test were analyzed

Markers	Proportion of plants exhibiting the markers		
	Population 1 (29 seedlings)	**Population 2 (27 seedlings)**	**Population 1 and 2 (56)**
AGC-CTG-d	96·5	100	98·2
AGC-CAT-a	96·5	96·3	96·4

The microsatellite data (Table 3) showed no strict selection for the homozygous introgressed genotypes in these resistant plants compared to the heterozygous ones, indicating the likelihood of a dominant expression of the gene *Ck*-1. However in Pop 1, the ratios of homozygous to heterozygous genotypes had χ^2 values significantly deviating from a 1:2 ratio that would be expected for strict dominance. This agreed with phenotypic data obtained during screening of these plants (Fig. 1). In G2 plants, the segregation of the markers in the total population (Table 3) fitted the expected Mendelian ratios of 1:2:1, confirming the absence of segregation distortion. After screening for CBD resistance there was apparent, though not significant, selection against plants without the introgressed alleles in the resistant category. Moreover, significant selection for plants without the introgressed alleles was observed in the susceptible category.

Table 3. Chi squared test on the segregation ratios of two microsatellite markers analysed in two F_2 populations from crosses between *Coffea arabica* cv. SL28 and cv. Catimor, lines 127 (Population 1) or 88 (Population 2), screened for coffee berry disease (CBD) resistance by inoculation with *Colletotrichum kahawae* at two stages of development. A + sign denotes the presence of the introgressed alleles while 0 denotes the presence of the SL28 allele. The observed marker segregations were compared to the expected distribution assuming either a Mendelian segregation ratio 1(++) : 2(+0): 1(00) or the expected ratio 1(++) : 2(+0) for a dominant marker linked with CBD resistance

Screening stage	Plant Pop.	Phenotypic reaction to infection	Marker	F_2 genotype ++	+0	00	Ratio expected	χ^2 value	*P*-value
Hypocotyl (G1)	1	Resistant	Sat 207	15	13	1	1:2:1	13·83	0·001
				15	13		1:2	4·29	0·0383
			Sat 235[a]	17	10	0	1:2:1	23·22	< 0·0001
				17	10		1:2	9·38	0·0022
	2	Resistant	Sat 207	10	15	2	1:2:1	5·07	0·0793
				10	15		1:2	0·24	0·6242
			Sat 235	12	15	0	1:2:1	11·00	0·0041
				12	15		1:2	1·04	0·3078
Young seedlings (G2)	2	Resistant	Sat 207	13	29	5	1:2:1	5·30	0·0707
			Sat 235	15	26	6	1:2:1	3·98	0·1367
		Susceptible	Sat 207	0	5	13	1:2:1	22·33	< 0·0001
			Sat 235	1	5	12	1:2:1	17·00	0·0002
		All plants[b]	Sat 207	21	48	26	1:2:1	0·54	0·7634

- [a] Failed amplification and treated as missing data for respective χ^2 calculations.
- b Includes plants of class 3 which were not categorized as either resistant or susceptible.

Table 3. Chi squared test on the segregation ratios of two microsatellite markers analysed in two F_2 populations from crosses between *Coffea arabica* cv. SL28 and cv. Catimor, lines 127 (Population 1) or 88 (Population 2), screened for coffee berry disease (CBD) resistance by inoculation with *Colletotrichum kahawae* at two stages of development. A + sign denotes the presence of the introgressed alleles while 0 denotes the presence of the SL28 allele. The observed marker segregations were compared to the expected distribution assuming either a Mendelian segregation ratio 1(++) : 2(+0): 1(00) or the expected ratio 1(++) : 2(+0) for a dominant marker linked with CBD resistance

Screening stage	Plant Pop.	Phenotypic reaction to infection	Marker	F_2 genotype			Ratio expected	χ^2 value	*P*-value
				++	+0	00			
			Sat 235	23	45	27	1:2:1	0·60	0·7408

Survey of CBD resistance markers in diverse accessions of Hibrido de Timor derivatives

Five lines derived from Hibrido de Timor, four resistant and one susceptible to CBD in field conditions in Kenya, were analysed with Sat 207 and Sat 235. All resistant plants were homozygous for the introgressed alleles at the Sat 235 locus while the susceptible one appeared not to be introgressed at the two loci. All the markers that were repeated in this phase were replicated as in the pre-screening and mapping stages.

Discussion

The hypocotyls-inoculation method has been used satisfactorily to select for CBD resistance, although there have been different opinions on scaling and data analysis (Van der Vossen *et al*., 1976; Van der Graaff, 1978, 1982; Dancer, 1986; Owour & Agwanda, 1990). In this study, a cut-off between presence and absence of resistance was made at class 10, based on the infection reaction of the susceptible control (cv. Caturra). The results fitted a 3:1 ratio assuming a major gene control ($P > 0{\cdot}05$). However, to obtain resistant sub-populations from the F_2 populations, the method adopted routinely in breeding programmes at CRF was used, whereby only seedlings in classes 1 to 4 are retained. This procedure had three advantages. First, these plants have higher chances of survival in the nursery than the more severely infected ones. Secondly, they have negligible chances of containing susceptible genotypes compared to the higher classes, although this risk is affected by the success of infection during screening – in this study, the cv. Caturra seeds were open pollinated, and therefore the two seedlings outside classes 11 and 12 might have been contaminations from the field or laboratory errors. Thirdly, by adopting the routine CRF procedure, the expected field resistance could be directly related to results of previous breeding programmes with the same degree of confidence as observed over time.

For mapping purposes, the hypocotyls-inoculation method (Van der Vossen *et al*., 1976) has the disadvantage of eliminating susceptible seedlings. The other method described by the same authors is the inoculation of seedlings with young shoot tips of 1–2 cm in length in an un-controlled environment (Van der Vossen *et al*., 1976). This implies that a population cannot be entirely screened at the same time, and the inoculations have to be done at particular periods of the year when environmental conditions are favourable. The young seedling-inoculation method developed in this study aimed to overcome the limitations of these screening methods, to facilitate screening and obtaining DNA from the entire populations. An acceptable separation of resistant and susceptible plants was achieved. Nevertheless, five seedlings (9·8%) of the susceptible cv. Caturra failed to be infected, and three (10·3%) of the resistant seedlings (G1) from Pop 1 reacted as susceptible. This demonstrated that some factors other than presence of resistance genes were involved. This can be explained by the occurrence of escapes and weak aneuploid plants even in pure lines of *C. arabica* (Van der Vossen & Walyaro, 1980). Based on the results of the two screening methods, the observed virulence of the pathogen on the susceptible control, and previous experiences of the hypocotyl screening method in CRF breeding programmes, G1 plants were considered to be truly resistant while some misclassification errors were expected in G2 plants due to lower accuracy of the shoot-tip test (Van der Vossen & Walyaro, 1980).

Eight AFLP and two microsatellite markers linked to CBD resistance were identified. They were mapped onto a chromosomal fragment derived from Hibrido de Timor. Results of G1 plants showed that the gene is located between Sat 235 and ACT-CAA-c, a distance of 10·6 cM, or just outside these markers. Further

refining of the map was not possible due to lack of recombinant plants of G1, but this could be possible when the G2 plants mature or by extensive survey of Hibrido de Timor derivatives with confirmed resistance to CBD. Results obtained from diverse accessions of Hibrido de Timor-derived lines demonstrated the reliability of identified microsatellite markers, and the resistance exhibited by various progenies of the Hibrido de Timor is due to a unique introgressed fragment.

The results of this study support the presence of a major gene for resistance. However, the results of both phenotypic and molecular analysis, particularly in G1 plants, suggests a lack of strict dominance and possible interaction with other genetic factors. This agrees with Van der Vossen & Walyaro (1980) who described CBD resistance in Hibrido de Timor to be of intermediate action controlled by one locus (*T*). Earlier, Van der Vossen *et al*. (1976) had speculated the action of minor genes even in susceptible varieties. In this study, seedlings with low vigour were found to be particularly vulnerable to infection and this raised doubts about whether vigour had some effect on the reaction to infection by *C. kahawae*. This may be due to weak plants that normally occur in Arabica coffee (Van der Vossen & Walyaro, 1980), and explain why the resistance has also been described as dominant based on evaluation of vigorous hybrid progenies, especially in the field (Omondi, 1994). Moreover, the differences of expressing resistance may be more pronounced in seedlings and less noticeable on berries of mature trees in the field. Resistance by plants to infection by *Colletotrichum* spp. is quite complex (Esquerré-Tugayé*et al*., 1992), including the coffee-*C. kahawae* interaction in particular (Gichuru, 1997; Gichuru *et al*., 1999).

The designation *Ck*-1 is suggested, as the first mapped locus of resistance to *C. kahawae*. This locus is most likely synonymous to the *T* locus described by Van der Vossen & Walyaro (1980). The possibility that other genetic factors in Hibrido de Timor-derived lines may play a role in CBD resistance (with or without pathogen specificity) is not ruled out. Identification of CBD-resistance markers is of great importance in breeding programmes, both in countries where the disease is present as well as where it is absent. Of particular importance are the highly repeatable and informative microsatellites (Sat 207 and Sat 235). The results of this work need to be complemented by similar work to identify markers for genes for CBD resistance and potentially new ones from other germplasm (e.g. Ethiopian accessions). This will help coffee breeders to distinguish the different CBD-resistance genes and also increase efficiency of gene stacking in future CBD-resistant cultivars.

Acknowledgements

This work was financed by IRD, France by a study fellowship to E.K.G., the European Union through CBDRESIST project (Contract No. ICA4-CT-2001-10008) and CRF, Kenya. Assistance from both technical and field staff of CRF and IRD is highly appreciated. This work is published with the permission of the Director of Research, CRF, Kenya.

References

- *Agwanda CO, Lashermes P, Trouslot P, Combes M-C, Charrier A, 1997. Identification of RAPD markers for resistance to Coffee Berry Disease, Colletotrichum kahawae in Arabica coffee. Euphytica 97, 241–8.*
    - CrossRef,
    - CAS,
    - Web of Science® Times Cited: 26
- *Bettencourt A, 1973. Consideraçoes gerais sobre o 'Hibrido de Timor'. Campinas, Brazil: Instituto Agronomico de Campinas, Circular No. 31.*
- *Combes MC, Andrzejewski S, Anthony F et al ., 2000. Characterization of microsatellite loci in Coffea arabica and coffee species. Molecular Ecology 9, 1178–80.*
  Direct Link:
    - Abstract
    - Full Article (HTML)
    - PDF(792K)
    - References
    - Web of Science® Times Cited: 30
- *Dancer J, 1986. The evaluation of arabica coffee cultivars for resistance to coffee berry disease: some comments. Euphytica 35, 125–6.*
    - CrossRef,
    - Web of Science® Times Cited: 2

- *Diniz LEC, Sakiyama NS, Lashermes P et al ., 2005. Analysis of AFLP markers associated to the Mex-1 locus in Icatu progenies. Crop Breeding and Applied Biotechnology 5, 387–93.*
  - CAS
- *Esquerré-Tugayé MT, Mazau D, Barthe JP, Lafitte C, Touzé A, 1992. Mechanisms of resistance to Colletotrichum species. In: BaileyJA, JegerMJ, eds. Colletotrichum; Biology, Pathology and Control. Wallingford, UK: CABI, 121–33.*
- *Gichuru EK, 1997. Resistance mechanisms in arabica coffee to coffee berry disease (Colletotrichum kahawae Sp. Nov.) – A review. Kenya Coffee 62, 2441–4.*
- *Gichuru EK, King'ori PN, Masaba DM, 1999. Histochemical differences during infection on Coffea arabica by Colletotrichum kahawae isolates. In: Proceedings of the 18th ASIC Colloquium, 1999. Helsinki, Finland. (Paper 717).*
- *Griffiths E, Gibbs JN, Waller JM, 1971. Control of coffee berry disease. Annals of Applied Biology 67, 45–74.*
  Direct Link:
  - Abstract
  - PDF(1924K)
  - References
  - Web of Science® Times Cited: 28
- *Lander SE, Green P, Abrahamson J et al ., 1987. Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genetics 1, 174–81.*
  - PubMed,
  - CAS
- *Lashermes P, Combes MC, Robert J et al ., 1999. Molecular characterization and origin of the Coffea arabica L. genome. Molecular and General Genetics 261, 259–66.*
  - CrossRef,
  - PubMed,
  - CAS,
  - Web of Science® Times Cited: 64
- *Lashermes P, Andrzejewski S, Bertrand B et al ., 2000a. Molecular analysis of introgressive breeding in coffee (Coffea arabica L.) Theoretical and Applied Genetics 100, 139–46.*
  - CrossRef,
  - CAS,
  - Web of Science® Times Cited: 40
- *Lashermes P, Combes MC, Topart P et al ., 2000b. Molecular breeding in coffee (Coffea arabica L.) In: SeraT, SoccolCR, PandeyA, RoussosS, eds. Coffee Biotechnology and Quality. Dordrecht, the Netherlands: Kluwer Academic Publishers, 101–12.*
- *Li YC, Korol AB, Fahima T, Beiles A, Nevo E, 2002. Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. Molecular Ecology 11, 453–65.*
  Direct Link:
  - Abstract
  - Full Article (HTML)
  - PDF(203K)
  - References
  - Web of Science® Times Cited: 213
- *Mahé L, Varzea VMP, Le Pierrès D, Combes MC, Lashermes P, 2007. A new source of resistance against coffee leaf rust from New-Caledonian natural interspecific hybrids between Coffea arabica and C. canephora. Plant Breeding 126, 638–41.*
  Direct Link:
  - Abstract
  - Full Article (HTML)
  - PDF(104K)
  - References
  - Web of Science® Times Cited: 1

- *Masaba DM, Waller JM, 1992. Coffee Berry Disease: the current status. In: BaileyJA, JegerMJ, eds. Colletotrichum; Biology, Pathology and Control. Wallingford, UK: CABI, 237–49.*
- *Nyoro JK, Sprey LH, 1986. Introducing Ruiru 11 to estates and smallholders. Kenya Coffee 51, 7–28.*
- *Omondi CO, 1994. Resistance to Coffee Berry Disease in Arabica Coffee Variety 'Ruiru 11'. Plant Breeding 112, 256–9.*
  Direct Link:
  - Abstract
  - PDF(2953K)
  - References
  - Web of Science® Times Cited: 2
- *Osorio N, 2002. The Global Coffee Crisis: A Threat to Sustainable Development. London, UK: International Coffee Organisation.*
- *Owour JBO, Agwanda CO, 1990. Comparison of the sensitivity of the mean and the mode in screening for resistance to coffee berry disease Colletotrichum coffeanum Noack sensu Hindorf (a short communication). Euphytica 45, 247–50.*
- *Rafalski JA, Vogel JM, Morgante M, Powell W, Andre C, Tingey SV, 1996. Generating and using DNA markers in Plants. In: BirenB, Lai, E, eds. Non-Mammalian Genomic Analysis: A Practical Guide. New York, USA: Academic Press Inc., 75–134.*
- *Raina SN, Mukai Y, Yamamoto M, 1998. In situ hybridization identifies the diploid progenitors of Coffea arabica (Rubiaceae). Theoretical and Applied Genetics 97, 1204–9.*
  - CrossRef,
  - Web of Science® Times Cited: 30
- *Rieseberg LH, Baird SJE, Gardner KA, 2000. Hybridization, introgression and linkage evolution. Plant Molecular Biology 42, 205–24.*
  - CrossRef,
  - PubMed,
  - CAS,
  - Web of Science® Times Cited: 93
- *Van der Graaff NA, 1978. Selection for resistance to coffee berry disease in arabica coffee in Ethiopia. Evaluation of selection methods. Netherlands Journal of Plant Pathology 84, 205–15.*
  - CrossRef
- *Van der Graaff NA, 1982. The principles of scaling and the inheritance of the coffee berry disease in Coffea arabica. Euphytica 31, 735–40.*
  - CrossRef
- *Van der Vossen HAM, Walyaro DJ, 1980. Breeding for resistance to coffee berry disease in Coffea arabica L. Inheritance of the resistance. Euphytica 29, 777–91.*
  - CrossRef
- *Van der Vossen HAM, Cook RTA, Murakaru GNW, 1976. Breeding for resistance to coffee berry disease caused by Colletotrichum coffeanum Noack sensu Hindorf in Coffea arabica L. I. Methods of preselection for resistance. Euphytica 25, 733–56.*
  - CrossRef
- *Vos P, Hoger R, Bleeker M et al ., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23, 4407–17.*
  - CrossRef,
  - PubMed,
  - CAS,
  - Web of Science® Times Cited: 5267

Get PDF (205K)

More content like this

Find more content:

- like this article

Find more content written by:

- E. K. Gichuru

- C. O. Agwanda
- M. C. Combes
- E. W. Mutitu
- E. C. K. Ngugi
- B. Bertrand
- P. Lashermes
- All Authors
- ABOUT US
- HELP
- CONTACT US
- AGENTS
- ADVERTISERS
- MEDIA
- PRIVACY
- COOKIES
- TERMS & CONDITIONS
- SITE MAP