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Research Paper

Occurrence of *Ck-1* gene conferring resistance to Coffee Berry Disease in *Coffea arabica* cv. Ruiru 11 and its parental genotypes

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Abstract. Resistance to Coffee Berry Disease (CBD) in *Coffea arabica* cv. Ruiru 11 is known to be controlled by among others, the T (*Ck-1*) gene from Robusta coffee (*Coffea canephora* Pierre). The cultivar reportedly presents significant variability in resistance to CBD. Previous work identified a microsattelite marker Sat 235 which was linked to CBD resistance and mapped it onto the introgressed *C. canephora* fragment which carries the *Ck-1* gene. This study was aimed at utilizing the Sat 235 marker to assess the occurrence of the *Ck-1* gene in Ruiru 11 sibs and their parental genotypes. The test genotypes used were CBD resistant Robusta coffee, non introgressed *C. arabica* cv. caturra, 14 Ruiru 11 parental genotypes with varying reaction to CBD and 34 Ruiru 11 sibs. Evaluation of CBD resistance was conducted in the laboratory using hypocotyl inoculation method. Seeds of the test genotypes were sown in plastic boxes filled to half-depth with sterilized river sand and arranged in a completely randomized design with three replications. Six weeks after sowing, the seedling hypocotyls were inoculated with a conidial suspension of *C. kahawae* standardized to 2.0×10^6 spores/ml. Disease scoring was conducted 4 weeks after inoculation on a scale of 1 to 12. To confirm occurrence of the *Ck-1* gene, genomic DNA was then extracted from the test genotypes and amplified with the microsatellite primer Sat 235 and electrophoresed on a 6% denaturing polyacrylamide gel. All the genotypes containing the *Ck-1* gene were expected to show phenotypic resistance to CBD and to show similar banding pattern as Robusta and HDT while the ones lacking the gene were expected to show phenotypic susceptibility to CBD and to similar banding pattern as Caturra and SL28. The study observed that all Ruiru 11 sibs that were evaluated contained the *Ck-1* gene. The study also provided further evidence that the fragment amplified by SSR primer Sat 235 is linked to CBD resistance.

Keywords: Coffee, introgression, molecular markers, Sat 235.

INTRODUCTION

Coffee Berry Disease (CBD) is an anthracnose caused by *Colletotrichum kahawae*, Waller and Bridge (Gichuru et al., 2008). The disease mainly infects the green immature berries, a stage in which it can cause up to 80% crop loss if not controlled (Gichimu and Phiri, 2010). Differences in resistance of coffee trees to CBD are frequently observed under field and laboratory conditions (Silva et al., 2006; Gichuru, 2007). In Kenya, varieties like Geisha 10, Blue Mountain and K7 were recommended for commercial growing due to their tolerance to CBD and Coffee Leaf Rust (CLR) that allowed acceptable yields to be realised without spraying (Silva et al., 2006; Gichuru, 2007). High levels of resistance were found in Rume Sudan and some progenies of Hibrido de Timor (HDT) (Silva et al., 2006). Rume Sudan resulted from seeds accessed from the Boma plateau in Sudan (Walyaro, 1983). HDT is a spontaneous inter-specific cross between *C. arabica* and *C. canephora* that originated on the island of Timor. Progenies of HDT and advanced inbred lines of its cross to *C. arabica* cv. Caturra (referred to as cv. Catimor),

are used as donor parents for resistance to CBD and CLR in Kenya (Gichuru et al., 2008).

Studies carried out in Kenya by Van der Vossen and Walyaro (1980) concluded that coffee resistance to CBD appears to be controlled by major genes on three different loci. The highly resistant variety Rume Sudan carries the dominant R- and the recessive k-genes. The moderately resistant variety K7 carries only the recessive k-gene (Agwanda et al., 1997; Silva et al., 2006; Van der Vossen, 2006). Clone 1349/269 of the variety HDT which was introduced in Kenya in 1960 from Portugal and its hybrid derivative Catimor carries one gene for CBD resistance on the T-locus with intermediate gene action (Van der Vossen and Walyaro, 1980; Omondi et al., 2001; Silva et al., 2006; Gichuru et al., 2008). Catimor has several iso-lines (Catimor 86, Catimor 88, Catimor 90, Catimor 124, Catimor 127 and Catimor 134) which are F3 and F4 progenies from a cross between HDT (CIFC accession number 1343) and *C. arabica* cv Caturra (Omondi et al., 2001). The iso-lines were introduced to Kenya from Colombia in 1975 and 1977 as seeds from single trees and each seed lot had a number that signified its lineage hence designations (Gichuru, 2007).

Introduction of resistance genes into susceptible varieties involves crossing with donor varieties, followed by backcrossing to restore desirable traits, especially yields and quality (Gichuru et al., 2008). The seedling hypocotyls inoculation method developed by Van der Vossen et al. (1976) shortened the period required to detect resistance to CBD. However, if conventional breeding methods are applied the breeding may take long especially when the programme requires procedures such as backcrossing (Anthony and Lashermes, 2005). The time required for breeding by traditional method can be shortened by use of DNA based marker assisted selection (MAS) (Rieseberg et al., 2000). The markers help in detecting a targeted genomic fragment and therefore selects for a desirable trait that is linked to it such as disease resistance, and this can be done in the early stages of plant growth (Gichuru et al., 2008). Selection by use of molecular markers results in a gain of about two generations of backcrossing and this gain can be higher if the objective is to reduce linkage drag (Riesenbierg et al., 2000). Agwanda et al. (1997) identified randomly amplified polymorphic DNA (RAPD) markers associated with CBD resistance derived from HDT but their use is limited by low reproducibility. Microsatellites, also known as simple sequence repeats (SSR) present the advantages of wide genome coverage, reproducibility, large number of data points developed in one reaction and high information content (Li et al., 2002; Gichuru et al., 2008).

The Kenyan Arabica composite cultivar Ruiru 11 is one of the cultivars whose major source of CBD resistance is Robusta coffee introgressed through HDT and Catimor (Gichuru, 2007). The cultivar is a composite of 66 F1 hybrid sibs each derived from a cross between a specific female and male population (Omondi et al., 2001). The male parents are outstanding selections from a multiple cross programme involving Coffee Berry Disease (CBD) resistant donor parents such as Rume Sudan (R gene), HDT (T gene), K7 (k gene) and the high yielding, good quality but susceptible cultivars such as N39, SL28, SL34, Bourbon and SL4 (Omondi et al., 2000). The female parents are advanced generations (F3 and F4) of the cultivar Catimor from Colombia, which has HDT clone 1343/269 as one parent (Omondi et al., 2000). The cultivar combines resistance to major CBD and CLR with high yield, fine quality and compact growth amenable to high density planting (Omondi et al., 2001). However, Omondi et al. (2001) observed that Ruiru 11 sibs present significant variability in resistance to CBD. Intra-selection within Ruiru 11 cultivar for resistance to CBD is therefore desirable. Using F2 plants (cv Catimor × cv SL28) that were resistant and susceptible to CBD, Gichuru (2007) identified a microsattelite marker Sat 235 which was linked to CBD resistance and mapped it onto an introgressed *C. canephora* fragment which carries the responsible *Ck-1* gene.

The objective of this study was to utilize the Sat 235 marker to assess the occurrence of the *Ck-1* gene in Ruiru 11 sibs and their parental genotypes.

MATERIALS AND METHODS

Test genotypes

The test genotypes used were Robusta coffee (*C. canephora* Pierre), non introgressed *C. arabica* cv. caturra, 14 Ruiru 11 parental genotypes (Table 1) and 34 Ruiru 11 hybrid sibs (Table 2).

Three new lines (CR8, CR22 and CR30) selected from Ruiru 11 male parents but selfed to fix the genes were also included in the study. The parentage of the three lines is as follows: CR8 = SL28 × [(SL34 × RS) HDT]; CR22 = SL28 × [(N39 × HDT) (SL4 × RS)]; CR30 = SL28 × [(K7 × RS) (SL34 × HDT)].

Laboratory evaluation of CBD resistance

Seeds of the Ruiru 11 hybrid sibs and their parental genotypes were obtained from the Plant Breeding Section of Coffee Research Station in Ruiru. The seeds were dehusked before sowing to enhance germination. The experiment was arranged in the laboratory in a completely randomized design (CRD) with three replications. For each of the test genotype, 150 seeds per replicate were sown in pregermination plastic boxes measuring 15 cm wide, 22 cm long and 15 cm deep filled to half-depth with sterilized river sand. Watering was done twice a week to ensure that the sand remained moist but

Table 1. Ruiru 11 parental genotypes and their reaction to CBD.

Serial no.	Ruiru 11 parent	Status	Reaction to CBD
1.	HDT	Breeder's material	Resistant (T gene)
2.	Catimor 86	Breeder's material	Resistant (T gene)
3.	Catimor 88	Breeder's material	Resistant (T gene)
4.	Catimor 90	Breeder's material	Resistant (T gene)
5.	Catimor 124	Breeder's material	Resistant (T gene)
6.	Catimor 127	Breeder's material	Resistant (T gene)
7.	Catimor 134	Breeder's material	Resistant (T gene)
8.	Rume Sudan	Wild Accession	Resistant (R gene)
9.	K7	Commercial cultivar (Kenya)	Tolerant (k gene)
10.	SL28	Commercial cultivar (Kenya)	Susceptible
11.	SL34	Commercial cultivar (Kenya)	Susceptible
12.	SL4	Breeder's material	Susceptible
13.	N39	Commercial cultivar (Tanzania)	Susceptible
14.	Bourbon	Breeder's material	Susceptible

not water logged. The seeds germinated into hypocotyl seedlings with unopened cotyledons 6 weeks after sowing. They were all uprooted and 100 seedlings selected and immediately replanted in the same boxes at a spacing of 2.5 cm × 2.5 cm.

The CBD inoculum was obtained from the Plant Pathology Section of Coffee Research Station in Ruiru. The inoculum was a conidial suspension of *C. kahawae* prepared from 10 days old cultures standardized to 2.0 × 10^6 spores/ml. The seedling hypocotyls were inoculated in the boxes twice at 48 h interval by spraying them with the inoculum using a hand sprayer. After every inoculation, the seedlings were incubated in the dark by covering them with black polythene sheet for 48 h at room temperature (22 to 24°C) and then transferred into a temperature controlled room at 18 ± 2°C for 2 weeks. They were then transferred back to room temperature for one more week, after which the symptoms were scored. The first experiment was conducted in July 2010 and repeated in July 2011.

CBD scoring

The CBD reaction scores were on a scale of 1 to 12 developed by Van der Vossen et al. (1976) as follows: 1 = No visible symptoms; 2 = A few scab lesions; 3 = Small scab or tiny brown lesions; 4 = Scab or brown lesions; 5 = Scab and brown lesions, and a few small black lesions; 6 = Brown and narrow black lesions; 7 = Narrow black lesions, some more than 1 centimetre long; 8 = Black lesions becoming wider and starting to coalesce; 9 = Large coalescing black lesions but not yet complete; 10 = Large coalescing black lesions, complete girdling of stem; 11 = Most of the stem affected, more than one third stem shrivelled, seedling dead; 12 = Whole stem affected and shrivelled, seedling dead (Figure 1). All the seedlings were individually scored and average infection (AI) on each genotype was calculated in each replication as follows:

$$AI = 1/N\sum_{i=1}^{12} in_i$$

where, ***i*** is the disease class;
n_i is the number of seedlings in class *i*;
N is the total number of seedlings scored (Van der Vossen et al., 1976).

Data analysis

The data were subjected to analysis of variance (ANOVA) using XLSTAT version 2012 software at 5% level of significance. Least Significance Difference ($LSD_{5\%}$) was used to separate the means.

Confirmation of occurrence of the T (*Ck-1*) gene

Extraction of genomic DNA

Young coffee leaves were picked from the growing tips of the test genotypes and lyophilized at least 72 h before extraction. The lyophilized leaves were stored at -21°C before DNA extraction. Genomic DNA was then extracted from the lyophilized leaves following the CTAB method (Diniz et al., 2005) with minor modifications. 500 mg of the lyophilized leaves were macerated in liquid nitrogen. 1 ml each of lysis and extraction buffers (Appendix 1a) was added to the powder in the mortar. The macerated tissue was distributed in two 1.5 ml tubes and incubated at 65°C in a water bath for 20 to 30 min with regular

Table 2. Pedigree of Ruiru 11 sibs evaluated.

Serial no.	Sibs	Parentage
1.	R11-1	CAT.86 x [SL28 x B3.96 = (SL28 x RS) (B x HDT)]
2.	R11-3	CAT.90 x [SL28 x B3.96 = (SL28 x RS) (B x HDT)]
3.	R11-5	CAT.124 x [SL28 x B3.96 = (SL28 x RS) (B x HDT)]
4.	R11-6	CAT.127 x [SL28 x B3.96 = (SL28 x RS) (B x HDT)]
5.	R11-7	CAT.128 x [SL28 x B3.96 = (SL28 x RS) (B x HDT)]
6.	R11-11	CAT.86 x [SL28 x B3.97 = (SL28 x RS) (B x HDT)]
7.	R11-22	CAT.88 x [SL28 x B3.99 = (SL28 x RS) (B x HDT)]
8.	R11-23	CAT.90 x [SL28 x B3.99 = (SL28 x RS) (B x HDT)]
9.	R11-41	CAT.86 x [SL28 x B3.116 = (SL28 x RS) (B x HDT)]
10.	R11-42	CAT.88 x [SL28 x B3.116 = (SL28 x RS) (B x HDT)]
11.	R11-50	CAT.134 x [SL28 x B3.116 = (SL28 x RS) (B x HDT)]
12.	R11-52	CAT.88 x [SL28 x B3.185 = (K7 x RS) (SL34 x HDT)]
13.	R11-71	CAT.86 x [SL28 x B3.314 = (N39 x HDT) (SL4 x RS)]
14.	R11-72	CAT.88 x [SL28 x B3.314 = (N39 x HDT) (SL4 x RS)]
15.	R11-80	CAT.134 x [SL28 x B3.314 = (N39 x HDT) (SL4 x RS)]
16.	R11-91	CAT.86 x [SL28 x B3.863 = (SL34 x RS) HDT]
17.	R11-93	CAT.90 x [SL28 x B3.863 = (SL34 x RS) HDT]
18.	R11-100	CAT.134 x [SL28 x B3.863 = (SL34 x RS) HDT]
19.	R11-103	CAT.90 x [SL28 x B3.866 = (SL34 x RS) HDT]
20.	R11-105	CAT.124 x [SL28 x B3.866 = (SL34 x RS) HDT]
21.	R11-106	CAT.127 x [SL28 x B3.866 = (SL34 x RS) HDT]
22.	R11-107	CAT.128 x [SL28 x B3.866 = (SL34 x RS) HDT]
23.	R11-111	CAT.86 x [SL28 x B3.886 = (SL34 x RS) HDT]
24.	R11-112	CAT.88 x [SL28 x B3.886 = (SL34 x RS) HDT]
25.	R11-115	CAT.124 x [SL28 x B3.886 = (SL34 x RS) HDT]
26.	R11-117	CAT.128 x [SL28 x B3.886 = (SL34 x RS) HDT]
27.	R11-121	CAT.86 x [SL28 x B3.887 = (SL34 x RS) HDT]
28.	R11-123	CAT.90 x [SL28 x B3.887 = (SL34 x RS) HDT]
29.	R11-125	CAT.124 x [SL28 x B3.887 = (SL34 x RS) HDT]
30.	R11-131	CAT.86 x [SL28 x B3.879 = (SL34 x RS) HDT]
31.	R11-135	CAT.124 x [SL34 x B3.879 = (SL34 x RS) HDT]
32.	R11-137	CAT.128 x [SL34 x B3.879 = (SL34 x RS) HDT]
33.	R11-142	CAT.88 x [SL28 x B4.54 = SL28 (SL28 x RS)]
34.	R11-143	CAT.90 x [SL28 x B4.54 = SL28 (SL28 x RS)]

Key: RS = Rume Sudan, HDT = Hibrido de Timor, CAT = Catimor, B = Bourbon.

shaking. After incubation, 1 ml of chloroform/isoamyl-alcohol mixture in the ratio of 24:1 was added to each tube, then mixed gently by shaking and centrifuged at 13000 rpm for 10 to 15 min in a micro-centrifuge. The supernatants were pipetted out into new 1.5 ml tubes. 20 µl of RNase were added to the supernatants and incubated at 37°C in a water-bath for 30 min. An equal volume of isopropyl alcohol was added into each tube and mixed gently by inverting the tubes several times to precipitate DNA. The suspended DNA was centrifuged at 13000 rpm for 5 min to obtain a DNA pellet and the supernatant carefully removed. The DNA pellets were then washed with 200 µl of 70% ethanol and centrifuged at 13000 rpm for 3 min. The ethanol was drained by decanting or micro-pipetting and the pellets dried in a vacuum centrifuge for 20 min. The pellets were dissolved in 20 to 40 µl of Tris-EDTA (TE) buffer (depending on pellet size) and stored at 4°C. DNA was then quantified using spectrophotometer.

SSR analysis

Extracted genomic DNA was amplified using microsatellite primer Sat 235 which is linked to *Ck-1* gene for CBD resistance. The primer was designed by Invitrogen, USA and has forward and reverse sequences of TCGTTCTGTCATTAAATCGTCAA and GCAAATCAT-GAAAATAGTTGGTG respectively. The analysis was conducted using the methodology described by Combes

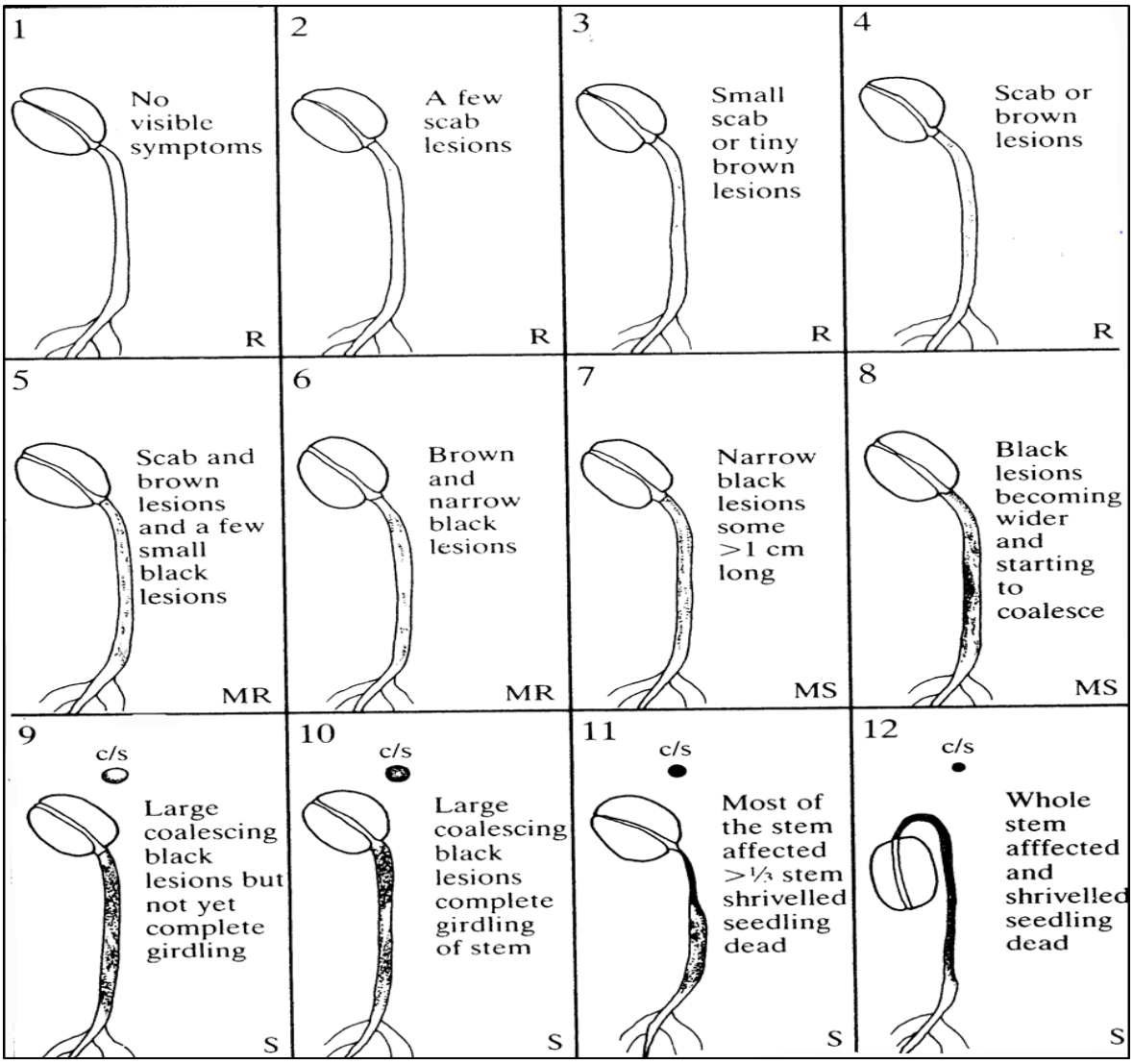

Figure 1. Sketch diagrams of the scoring system (Classes 1 to 12) of coffee seedling hypocotyls after inoculation with *C. kahawae* inoculum as described by Van der Vossen et al. (1976) (Source: Gichuru, 2007). Key: R: Resistant; MR: Medium Resistant; MS: Medium Susceptible, S: Susceptible; c/s: cross section.

et al. (2000) with minor modifications. Amplification was in 25 µl PCR reaction mix (Appendix 1b) containing 2.3 µl of double distilled water, 5 µl of 10 ng/µl genomic DNA, 2.5 µl of 10X PCR buffer (Promega), 2.5 µl of $MgCl_2$ (25 mM, Promega), 7.5µl of SSR dNTPs (250µM, Eurogentec), 2.5 µl each of forward and reverse Sat 235 primer (10 µM, Eurogentec), 0.2µl of Taq DNA polymerase (5U/µl, Promega). The PCR programme consisted of an initial denaturation of 5 min at 94°C followed by 5 cycles of 45 s of denaturation at 94°C, 90 s primer annealing at 60°C reducing by 1°C every cycle, elongation for 2 min at 72°C and 35 cycles of 30 s of denaturation at 94°C, primer annealing at 54°C for 60 s and elongation at 72°C for 90 s and final extension of 10 min at 72°C. 8 µl of loading dye was added to the amplified sample.

Amplification products were electrophoresed on a 6% denaturing polyacrylamide gel consisting of 27 g urea, 9 ml of Bis-acrylamide (40%), 12 ml of TBE (5X) and 60 ml of double distilled water (Appendix 1c). The machine was allowed to pre-run for 1 h with power supply set at 1100 V, 110 mA and 110 W to attain a temperature of 45 to 50°C. 10 µl of amplification products were loaded in the sample wells and electrophoresed for 3 h. Bands were visualized and photographed on a White Light Transilluminator after silver staining. The candidates containing the T (*Ck-1*) gene were expected to share common or similar bands with Robusta and Hibrido de Timor (HDT) and to show phenotypic resistance to CBD. On the other hand, susceptible candidates without the *Ck-1* such as SL28 were expected to share common or similar bands with the non-introgressed cultivar, Caturra.

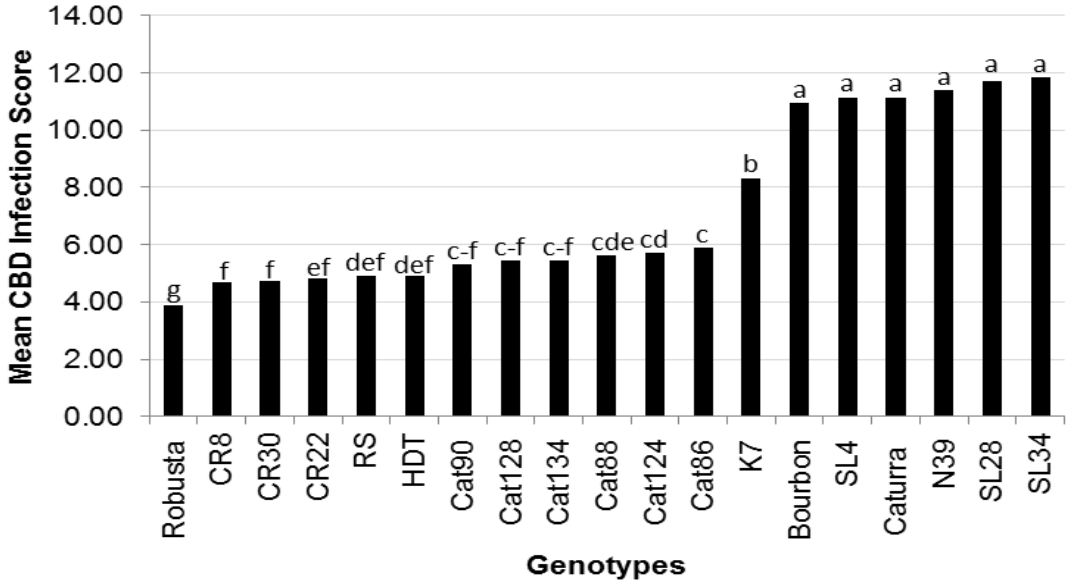

Figure 2. Mean CBD infection score in Robusta, Caturra and 14 Ruiru 11 parental genotypes.

RESULTS

Laboratory evaluation of CBD resistance

Results of inoculation of Robusta and Ruiru 11 parental genotypes with CBD inoculum are illustrated in Figure 2. The genotypes demonstrated significant ($p < 0.05$) variation in their reaction to CBD infection. There were no genotypes in the resistant (score 1 to 3) class. Robusta was the most resistant recording an average infection score of 3.88. Rume Sudan (RS), HDT, Catimor iso-lines (Cat86, 88, 90, 124, 128, 134) and the new lines (CR8, CR22 and CR30) were all in the moderate resistant class 4-6. Only K7 with an average infection score of 8.33 was in the moderate susceptible class 7 to 9. Bourbon, SL4, Caturra, N39, SL28 and SL34 were all susceptible (score 10 to 12).

Separate as well as combined analysis of variance for the two inoculation experiments showed that phenotypic variation of Ruiru 11 sibs in resistance to CBD was also significant ($p < 0.05$). Some sibs recorded varying results during the two screening experiments (Table 3). This might have been caused by differences in CBD inoculum since a different inoculum was prepared for each experiment though following the same protocol. The cultivar SL28 which was used as a susceptible control was the only genotype that was in the susceptible class (score 10 to 12) with average infection scores of 11.59 and 11.72 in first and second experiments respectively (Table 3). Resistance in Ruiru 11 sibs ranged from moderately resistant to moderately susceptible but none of them fell in the resistant (score 1 to 3) and susceptible (score 10 to 12) classes. The most resistant was R11-143 with average infection scores of 4.55 and 4.71 in first and second experiments respectively (Table 3). Other sibs that also recorded good resistance to CBD include R11-1, R11-3, R11-5, R11-22, R11-23, R11-42, R11-80, R11-93, R11-105, R11-107, R11-121 and R11-135 with average infection scores of 6.43, 5.85, 6.21, 6.46, 6.13, 6.17, 5.52, 6.26, 6.25, 6.49, 5.94 and 6.31 respectively. The rest of Ruiru 11 sibs were in the range of 7 to 9 (Table 3) and were therefore rated as moderately susceptible.

Confirmation of occurrence of the T (*Ck-1*) gene

Microsatellite primer Sat 235 obtained differential polymorphism between resistant and susceptible genotypes (Plate 1). Non-introgressed alleles of Sat 235 were observed in all susceptible genotypes namely Caturra, Bourbon, SL4, N39, SL28, SL34 and K7. On the other hand, introgressed alleles were variously present in all resistant parental genotypes except Rume Sudan which is not introgressed, one representative of Catimor 124 and two representatives each in the population of CR8 and CR22. The marker representing the introgressed allele (arrowed in plate 1) was therefore considered to be linked to the *Ck-1* gene (Figure 3). Although Ruiru 11 sibs portrayed varying degrees of phenotypic resistance to CBD, all the sibs evaluated were found to contain the introgressed allele (arrowed in Figures 4 and 5).

DISCUSSION

Variation in CBD resistance was observed among Ruiru 11 parental genotypes as well as among different sibs of Ruiru 11. This confirmed the report of Silva et al. (2006)

Table 3. Variation in CBD infection on Ruiru 11 sibs.

Genotypes	Mean CBD infection score		
	Experiment 1	Experiment 2	Combined
R11-1	7.33^{g-j}	5.55^{no}	6.43^{h-j}
R11-3	4.82^{rs}	6.89^{g-l}	5.85^{kl}
R11-5	6.15^{l-o}	6.27^{l-n}	6.21^{i-k}
R11-6	9.22^{b}	6.12^{l-n}	7.67^{de}
R11-7	7.45^{f-j}	7.83^{b-f}	7.64^{de}
R11-11	6.51^{k-m}	6.56^{j-m}	6.53^{g-i}
R11-22	6.40^{k-m}	6.53^{lm}	6.46^{g-j}
R11-23	8.13^{c-f}	4.14^{p}	6.13^{i-k}
R11-41	8.76^{bc}	8.56^{b-d}	8.66^{b}
R11-42	6.44^{k-m}	5.89^{mn}	6.17^{i-k}
R11-50	7.88^{e-h}	7.69^{d-g}	7.79^{d}
R11-52	8.14^{c-f}	7.46^{f-j}	7.80^{d}
R11-71	5.54^{o-q}	7.76^{c-g}	6.65^{f-i}
R11-72	7.63^{e-i}	6.71^{h-m}	7.17^{ef}
R11-80	6.54^{k-m}	4.51^{p}	5.52^{l}
R11-91	8.68^{b-d}	8.62^{bc}	8.65^{b}
R11-93	6.10^{l-p}	6.43^{l-n}	6.26^{h-k}
R11-100	9.22^{b}	8.46^{b-e}	8.84^{b}
R11-103	5.66^{n-q}	8.42^{b-e}	7.04^{fg}
R11-105	5.06^{q-s}	7.45^{f-k}	6.25^{h-k}
R11-106	6.31^{l-n}	7.73^{c-g}	7.02^{fg}
R11-107	5.38^{p-r}	7.59^{e-h}	6.49^{g-j}
R11-111	8.04^{d-g}	7.56^{e-i}	7.80^{d}
R11-112	7.29^{h-j}	8.69^{b}	7.99^{cd}
R11-115	8.23^{c-e}	8.57^{b-d}	8.40^{bc}
R11-117	7.49^{f-j}	6.89^{g-l}	7.18^{ef}
R11-121	6.03^{m-p}	5.84^{mn}	5.94^{j-l}
R11-123	8.63^{b-d}	6.75^{h-m}	7.69^{de}
R11-125	7.55^{e-i}	6.72^{h-m}	7.15^{ef}
R11-131	8.73^{b-d}	6.54^{k-m}	7.63^{de}
R11-135	5.94^{m-p}	6.66^{i-m}	6.31^{h-k}
R11-137	7.10^{i-k}	6.51^{lm}	6.80^{f-h}
R11-142	6.79^{j-l}	6.48^{lm}	6.63^{f-i}
R11-143	4.55^{s}	4.71^{op}	4.63^{m}
SL28	11.59^{a}	11.72^{a}	11.65^{a}

Means followed by the same letter(s) within the column are not significantly different at $P \leq 0.05$.
Key: The hyphen (-) represents the alphabetical range between the letters.

that differences in resistance of coffee trees to CBD are frequently observed under field and laboratory conditions. As expected, Robusta (*C. canephora*), which is known as the major source of resistance in Ruiru 11 (Omondi et al., 2001) was the most resistant to CBD among the parental genotypes. This resistance is introgressed to Ruiru 11 through HDT or Catimor (Gichuru, 2007) both of which were also found to be resistant as expected. Apart from Robusta, good phenotypic resistance to CBD was observed in Rume Sudan and the new lines (CR8, CR22 and CR30) selected from Ruiru 11 male parents but selfed to fix the genes.

CBD resistance in the new lines may be derived from Rume Sudan and HDT both of which are in their pedigree. Unlike the original male parents of Ruiru 11, these new lines have been selfed at least five times and they are therefore believed to be true breeding. The three lines were released to Kenyan farmers in 2010 as Batian 1 (CR8), Batian 2 (CR22) and Batian 3 (CR30). The CBD tolerant cultivar K7 fell in the moderate susceptible class. Similar results were observed by Omondi et al. (2000). The moderate resistance reaction in the Kent type commercial cultivar K7 is conferred by a recessive k-gene (Agwanda et al., 1997; Silva et al., 2006; Van der

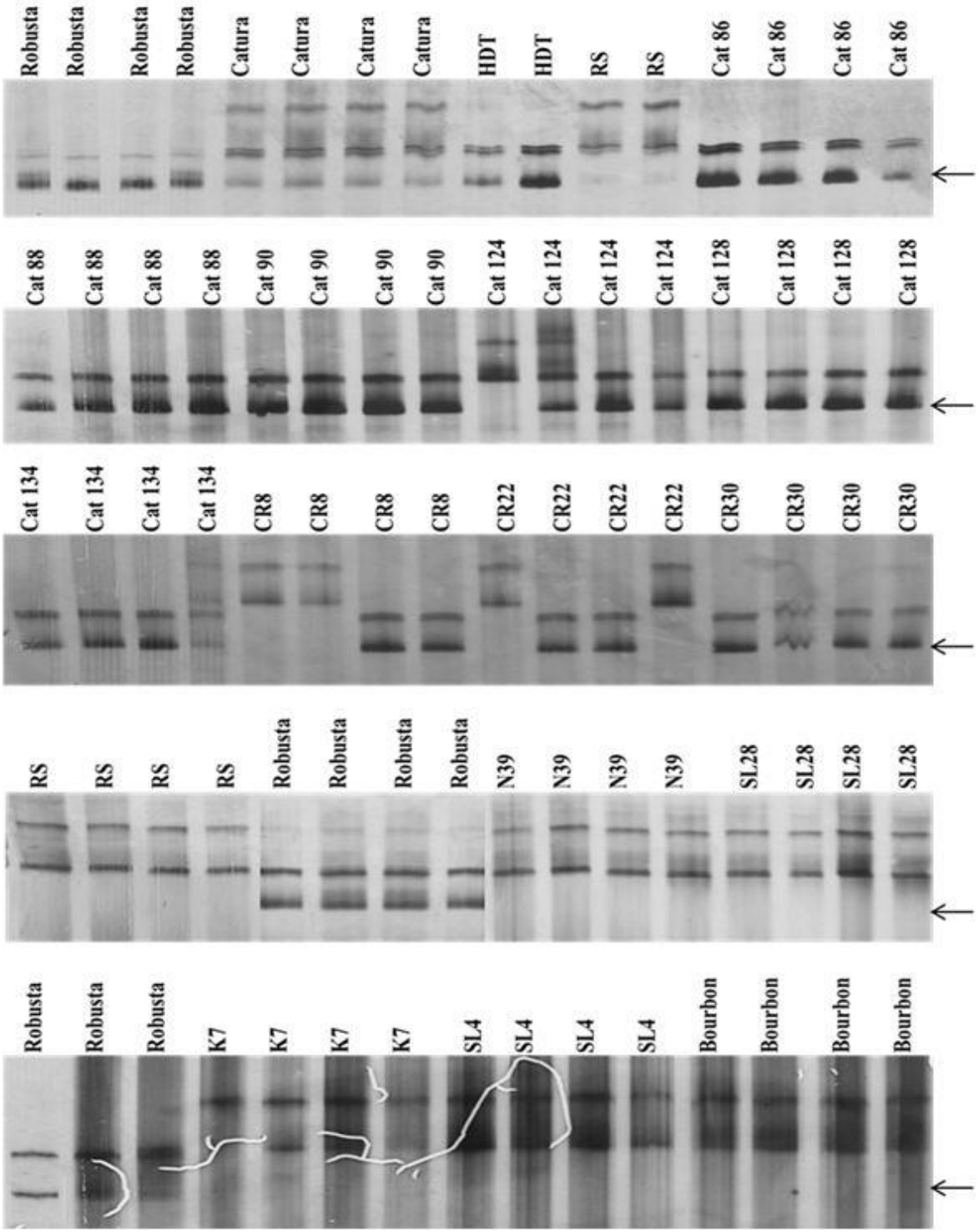

Figure 3. Banding patterns of Robusta, Caturra and Ruiru 11 parental genotypes generated by SSR primer Sat 235. Introgressed allele is arrowed.

Vossen, 2006). Silva et al. (2006) reported that 3-5 recessive genes control resistance to CBD in non-introgressed Arabicas. Other non-introgressed parental genotypes of Ruiru 11 namely SL28, SL34, SL4 and Bourbon are highly susceptible.

Occurrence of the T (*Ck-1*) gene was confirmed in all CBD resistant parental genotypes except Rume Sudan which is not introgressed, one representative of Catimor 124 and two representatives each in the population of CR8 and CR22. For Rume Sudan, CBD resistance is

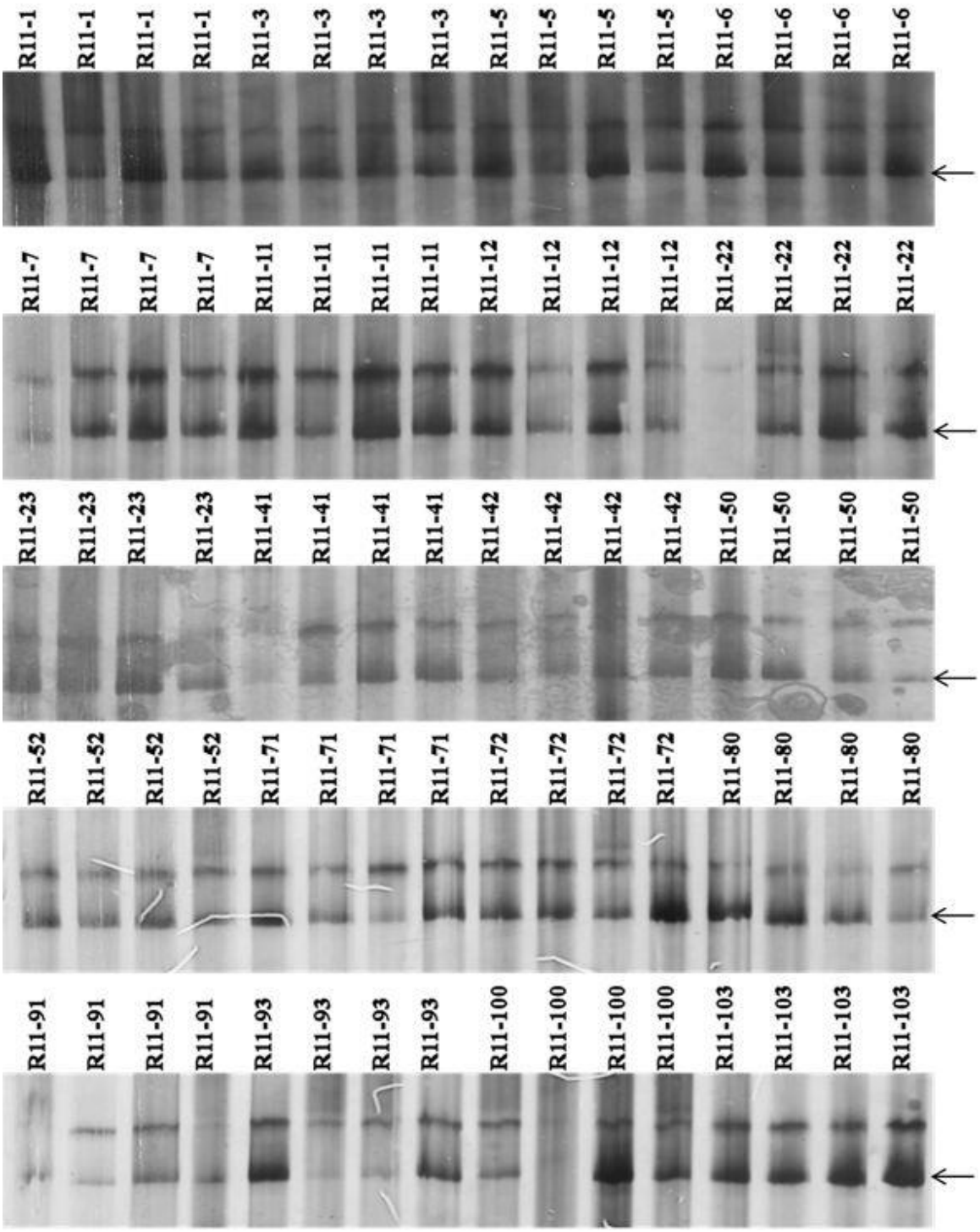

Figure 4. Banding patterns of Ruiru 11 sibs (set 1) generated by SSR primer Sat 235. Introgressed allele is arrowed.

conferred by a dominant R gene and a recessive k gene (Agwanda et al., 1997; Omondi et al., 2001; Silva et al., 2006; Van der Vossen and Walyaro, 2009) rather than the *Ck-1* gene. It therefore lacks the Sat 235 introgressed allele. Similarly, it may imply that the representative of Catimor 124 and the new lines that showed phenotypic resistance to CBD but failed to amplify the introgressed allele also contains only the Rume Sudan type of CBD resistance. Another possibility could be that the introgressed allele in these lines has been lost through

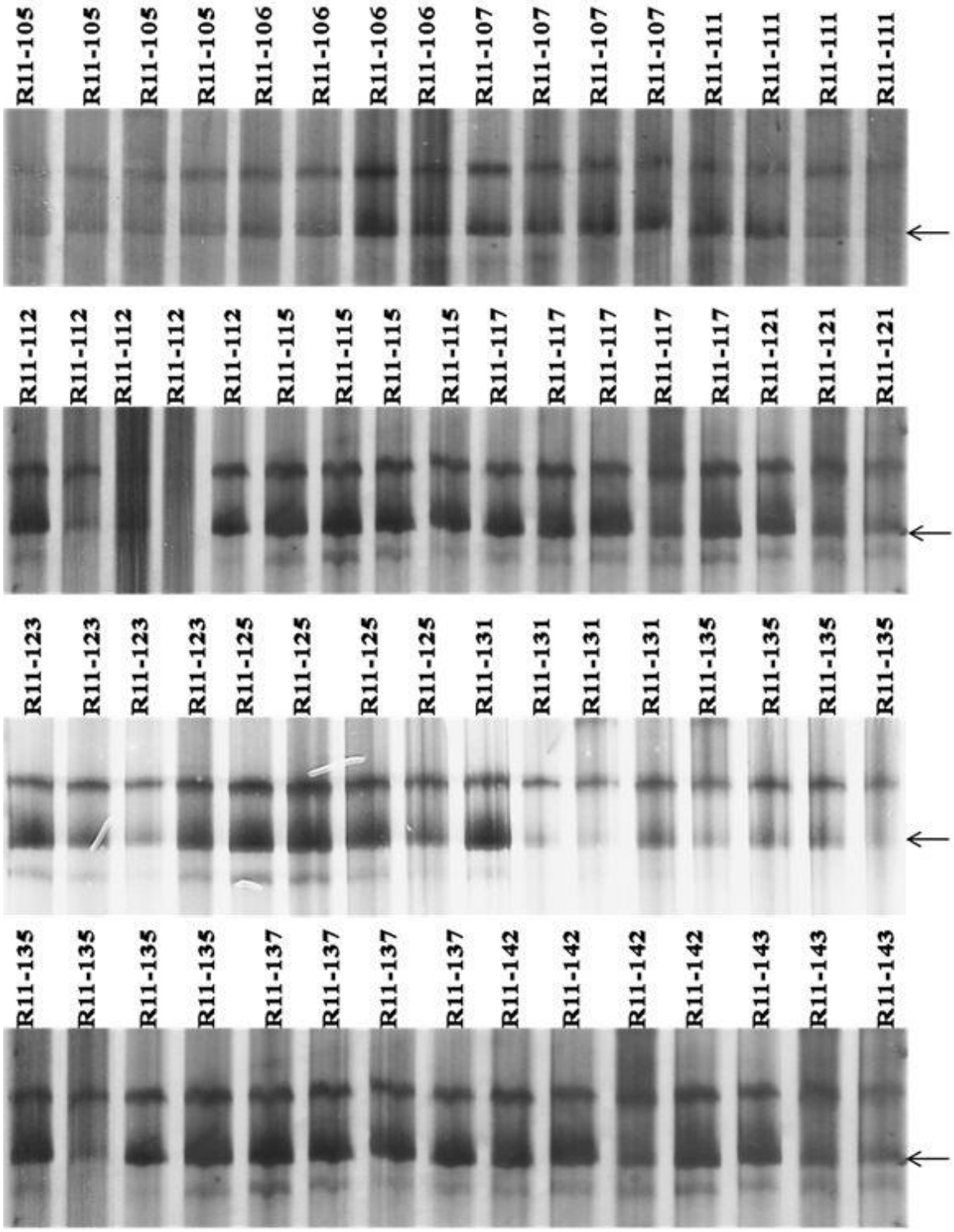

Figure 5. Banding patterns of Ruiru 11 sibs (set 2) generated by SSR primer Sat 235. Introgressed allele is arrowed.

genetic recombination. This study therefore showed similar findings to those by Gichuru (2007) thus providing further evidence that Sat 235 is linked to CBD resistance.

The observed variability in CBD resistance among Ruiru 11 sibs concurred with the findings of Omondi et al. (2001) that although the composite cultivar, Ruiru 11 generally contains good resistance to CBD, this resistance is not uniform among the sibs. Although these

Ruiru 11 sibs portrayed varying degrees of phenotypic resistance to CBD, all of them were found to contain the introgressed allele. Omondi et al. (2001) also reported that all the Ruiru 11 sibs should carry *Ck-1* gene from the Catimor mother parents and sometimes derived from male parents with HDT in their pedigrees. However, phenotypic variation in resistance was expected since apart from the *Ck-1* gene, the male parents may impart the R-gene of resistance if they are derived from crosses with Rume Sudan. Moreso, resistance to CBD carried by some male parents derived from crosses with Rume Sudan and HDT may not be expressed by some Ruiru 11 sibs because the male parents are not genetically fixed for resistance to CBD. This partly explains the variation in resistance which is observed among sibs with similar pedigrees. The third recessive k-gene is present in some male parents but is still lacking in the Catimor mother parents thus contributing further to observed variation.

CONCLUSION

The study confirmed that the *Ck-1* gene was present in all Ruiru 11 hybrid sibs that were evaluated. The gene was also present in all resistant parental genotypes except Rume Sudan which is not introgressed and two representatives each in the population of CR8 and CR22 whose CBD resistance could be conferred by the R gene. Although not all available Ruiru 11 sibs were evaluated, this was a good indication that the *Ck-1* gene could be present in all available Ruiru 11 sibs. The study also provided further evidence that the fragment amplified by SSR primer Sat 235 is linked to CBD resistance. This was a further confirmation of the potential use of Sat 235 as a molecular marker for CBD resistance in marker assisted selection. Thirteen Ruiru 11 sibs with good resistance to CBD were identified namely R11-143, R11-1, R11-3, R11-5, R11-22, R11-23, R11-42, R11-80, R11-93, R11-105, R11-107, R11-121 and R11-135. These sibs are recommended to farmers in CBD prone agro-ecological zones especially on higher altitudes. The sibs can also be exploited in future breeding programmes.

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REFERENCES

Agwanda CO, Lashermes P, Pierre T, Combes MC, Charrier A (1997). Identification of RAPD markers for resistance to coffee berry disease, *Colletotrichum kahawae*, in Arabica coffee Euphytica 97:241-248.

Anthony F, Lashermes P (2005). Origin, Evolution and Diversity of the coffee (*Coffea arabica* L.) genome. *In*: Sharma, A. K. and Sharma, A. (Eds), Plant genome: Biodiversity and evolution Vol. I (B). Science Publisher, Inc. Plymouth, UK. pp. 208-228.

Combes MC, Andrzejewski S, Anthony F, Bertrand B, Rovelli P, Graziosi G, Lashermes P (2000). Characterization of microsatellite loci in *Coffea arabica* and related coffee species. Mol. Ecol. 9:1178-1180.

Diniz LEC, Sakiyama NS, Lashermes P, Caixeta ET, Oliveiera ACB, Zambolin EM, Loureiro ME, Pereira AA, Zambolin L (2005). Analysis of AFLP markers associated to the *Mex*-1 locus in Icatu progenies. Crop Breed. Appl. Biotech. 5:387-393.

Gichimu BM, Phiri NA (2010). Response of Newly Developed and Introduced Arabica Coffee Genotypes to *Colletotrichum kahawae*, the Coffee Berry Disease Pathogen, in Kenya. Proceedings of 12th Biennial KARI Scientific Conference, Nairobi, Kenya, 2010.

Gichuru EK, Agwanda CO, Combes MC, Mutitu EW, Ngugi ECK, Bertrand B, Lashermes P (2008). Identification of molecular markers linked to a gene conferring resistance to coffee berry disease (*Colletotrichum kahawae*) in C*offea arabica.* Plant Pathol. 57:1117-1124.

Gichuru EK (2007). Characterization of genetic resistance to Coffee Berry Disease (*Colletotrichum kahawae* Waller and Bridge) in Arabica coffee (*Coffea arabica* L.) that is introgressed from *Coffea canephora* Pierre. PhD Thesis, University of Nairobi.

Li, YC, Korol AB, Fahima T, Beiles A, Nevo E (2002). Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. Mol. Ecol. 11:2453-2465.

Omondi CO, Ayiecho PO, Mwang'ombe AW, Hindorf H (2000). Reaction of some *Coffea arabica* genotypes to strains of *Colletotrichum kahawae*, the cause of coffee berry disease. J. Phytopathol. 148:61-63.

Omondi CO, Ayiecho PO, Mwang'ombe AW, Hindorf H (2001). Resistance of Coffea arabica cv. Ruiru 11 tested with different isolates of Colletotrichum kahawae, the causal agent of coffee berry disease. Euphytica 121:19-24.

Riesenbierg LH, Baird SJE, Gardner KA (2000). Hybridization, introgression and linkage evolution. Plant Mol. Biol. 42: 205-224.

Silva MC, Várzea V, Guerra-Guimarães L, Azinheira HG, Fernandez D, Petitot AS, Bertrand B, Lashermes P, Nicole M (2006). Coffee resistance to the main diseases: leaf rust and coffee berry disease. Braz. J. Plant Physiol. 18(1):119-147.

Van der Vossen HAM (2006). State-of-Art of developing Arabica coffee cultivars with durable resistance to coffee berry disease (*Colletotrichum kahawae*). Proc. 21st Int. Scient. Conf. on Coffee Sci., Montpellier, France pp. 794-801.

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 pre-selection for resistance. Euphytica 25:733-56.

Van der Vossen HAM, Walyaro DJ (2009). Additional evidence for oligogenic inheritance of durable host resistance to coffee berry disease (*Colletotrichum kahawae*) in arabica coffee (*Coffea arabica* L.). Euphytica 165:105-111.

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.

Walyaro DJ (1983). Considerations in breeding for improved yield and quality in arabica coffee (*Coffea arabica L.*). PhD Thesis, University of Wageningen, Netherlands.