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Resistance of *Coffea arabica* cv. Ruiru 11 tested with different isolates of *Colletotrichum kahawae*, the causal agent of coffee berry disease

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Summary

Seven single conidia isolates of *Colletotrichum kahawae* varying in pathogenicity were used to inoculate hybrid progenies from 66 crosses of *Coffea arabica* cv. Ruiru 11. The objective of this study was to investigate the effect of pathogen variation on resistance of the Ruiru 11 cultivar. The main effects of crosses and isolates were significant ($p \leq 0.05$) while their interaction effects were non-significant. Partitioning variance components indicated that the proportion of phenotypic variance for resistance that is due to genetic effects was low. It was concluded that variation for resistance among hybrid progenies of the Ruiru 11 cultivar was probably due to differences in aggressiveness of the pathogen as reflected by the significant main effects of crosses and isolates in combination with other environmental factors which influence disease epidemics. The coffee berry disease pathogen is unlikely to have adapted to the cultivar because of the non-significant crosses × isolates interaction effects.

Introduction

Coffee is an important export crop and a major foreign exchange earner for Kenya. It is the second most important agricultural commodity after tea, contributing upto 20% of the total hard currency revenue (Opile, 1993). It is further estimated that out of the 70% of Kenya's workforce engaged in agriculture, 30% are employed by the coffee industry. Over 90% of the total Kenya coffee acreage is under *Coffea arabica* L. The rest is occupied by *C. canephora* (Robusta coffee). Production of *C. arabica* is seriously constrained by diseases. The major diseases are coffee berry disease (*Colletotrichum kahawae*), coffee leaf rust (*Hemileia vastatrix*) and bacterial blight of coffee (*Pseudomonas syringae* pv. garcae). Coffee berry disease (CBD) which is a serious anthracnose of green and ripening berries causes major losses especially if the weather conditions are favourable to its epidemics. Control of the disease by fungicides is expensive and may account for upto 30% of the production costs (Nyoro & Sprey, 1986). It has also been reported that continuous use of Benzimidazole compounds was found to induce the emergence of fungicide-tolerant strains (Cook & Pereira, 1976; Okioga, 1976; Javed, 1980). The strains still persist in the pathogen population despite the fungicides having been withdrawn immediately the phenomenon was detected. (King'ori & Masaba, 1991; Mwang'ombe et al., 1992)

An economical and sustainable control may be achieved by growing resistant cultivars. An Arabica coffee cultivar, Ruiru 11, developed at the Coffee Research Station, Ruiru, Kenya, and released to growers in 1985, combines resistance to CBD and leaf rust with high yield, fine quality and compact growth amenable to high density planting. Inheritance studies using 11 *C. arabica* varieties varying in CBD resistance revealed three major genes of resistance on separate loci (Van der Vossen & Walyaro, 1980). The highly resistant variety, Rume Sudan carries the dominant R- and the recessive k-genes. The R-locus has multiple alleles with R_1R_1 in Rume Sudan and R_2R_2 in Pretoria, which also carries the k-gene. The moderately resistant variety K7 carries only 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. Pathogenicity tests performed with isolates from Ethiopia and Kenya detected no races but revealed that pathogen variation was mainly due to differences in aggressiveness (Van der Graaff, 1978; Masaba & Van der Vossen, 1980; Omondi et al., 2000). Although small but significant differential isolate × variety interactions have been reported, the contribution of the isolates to the interaction effect was found to be too small to suggest conclusively that races exist (Omondi et al., 2000). Further studies within *C. kahawae* at the DNA level have also revealed limited or total lack of polymorphism (Sreenivasaprasad et al., 1993; Beynon et al., 1995; Biratu, 1995; Omondi, 1998).

In a comparative study with isolates from Kenya, Angola and Malawi, Rodrigues Jr. et al. 1991 observed that the Kenyan strain had characteristics different from the Angolan and Malawian strains. Rodrigues Jr. et al. (1992) concluded that physiologic forms (races) of the CBD pathogen might exist among the Angolan, Malawian and Kenyan isolates. Beynon et al. (1995) and Manga et al. (1997) performed complementation tests with mutants of *C. kahawae* in the nitrate assimilation pathway and found several vegetative compatibility groups (VCGs). Formation of hyphal fusions which have been observed in *C. kahawae* is a likely mechanism by which genetic materials may be exchanged to create pathogen variation (Mwang'ombe et al., 1992). The objective of this study was therefore to determine the relative magnitude of cultivar, isolate and cultivar × isolate interaction effects and their implications on possible pathogen adaptation to the resistant Ruiru 11 cultivar.

Materials and methods

C. arabica cv. Ruiru 11 is a composite of hybrids obtained by crossing two sets of parents established at the seed garden of the Coffee Research Foundation, Ruiru, Kenya. The male parents are outstanding selections from a multiple cross programme involving CBD resistant donor parents such as Rume Sudan (RS), Hibrido de Timor (HT) and K7 and the high yielding, good quality but susceptible cultivars such as SL 28, SL 34, Bourbon (B) and a drought resistant selection (DRI). The female parents are advanced generations (F3, F4 and F5) of the cultivar Catimor, ex Colombia, which has Hibrido de Timor clone 1343/269 as one parent. Ruiru 11 hybrid progenies were produced from 66 crosses. Each cross was obtained through a hybridization programme using bulked pollen from up to 20 genetically similar clones of each male parent and several true-to-type progenies of each Catimor mother parent. The crosses were made during the October 1995–March 1996 seed production season.

Seven monoconidial isolates obtained from both resistant and susceptible cultivars across the range of coffee growing districts were used for inoculation. The isolates were designated according to the phenotype of the host cultivar, where, R-isolates were obtained from resistant Ruiru 11 cultivar and S-isolates were obtained from susceptible SL 28 cultivar. This was followed by a numerical code for the locality of origin separated from the serial number of the isolate with a point. The experiment was arranged in the laboratory in a completely randomized design with two replications. Each replicate was represented by 100 hypocotyl seedlings of the crosses and a line of 10 seedlings of the susceptible SL28 control pre-germinated alongside the crosses in a plastic box containing sterilized sand. The boxes measuring 15 cm wide, 22 cm long and 15 cm deep were filled to half-depth with sand. All seedlings were inoculated the same day with conidia suspensions from 10 days old cultures standardized to 2×10^6conidia/ml following the procedure of Van der Vossen et al. (1976). After three weeks, the seedlings were scored individually on a scale of 1 (no visible symptoms), to 12 (whole seedling dead). A mean grade of infection (G) was calculated for crosses in each replication as follows:

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

where, i is the disease class, n_i is the number of seedlings in class i and N is the total number of seedlings scored. The mean grade data were subjected to analysis of variance according to the following random effects model (Steel and Torrie, 1981): $Y_{ijk} = \mu + a_i + b_j + ab_{ij} + e_{k(ij)}$ where, Y_{ijk} = mean grade of isolate i/cross j combination in replication k, μ = overall mean, a_i = the effect of isolate i, b_j = the effect of cross j, ab_{ij} = the interaction effect of isolate i and cross j, and $e_{k(ij)}$ = the experimental error. The control seedlings were only used to observe pathogenicity of the isolates on the susceptible host. As required in random models, the isolates and crosses were random samples from their entire populations. Components of variance were estimated by equating observed mean squares to

their expectations. Estimates of phenotypic variance ($\sigma^2{}_p$) and broad sense heritability (H^2) for resistance were computed according to Falconer (1989) as follows: $\sigma^2{}_p = \sigma^2{}_c + \sigma^2{}_{cs} + \sigma^2{}_e$ and $H^2 = \sigma^2{}_c / \sigma^2{}_p$, where, $\sigma^2{}_c$ = variance due to crosses effects, $\sigma^2{}_{cs}$ = variance due to crosses × isolates interaction effects and $\sigma^2{}_e$ = error variance.

Results

The disease scores and the parentage of the Ruiru 11 crosses tested are presented in Table 1. The control seedlings were uniformly susceptible to all isolates with mean grade scores ranging from 10.50 to 12.00. These results were excluded from the analysis of variance. Twelve crosses were found in the resistant classes, 4–6 while majority fell in the medium resistant classes of 7—9 and only four crosses were in the susceptible classes of 10–12. There were no crosses in the highly resistant classes, 1–3. The parental mean scores were in the medium resistant classes of 7–9 except for the male parents, SL 28 × B4.691 = (DR1 × HT)(RS × SL 28) and SL 28 × B4.609 = (RS × SL 28)SL 28, which were in the resistant classes, 4–6. The two male parents were apparently under-represented in the crossing programme.

Results in Table 2 indicate that main effects of isolates differed significantly ($p \leq 0.05$) reflecting differences in aggressiveness. Variation among hybrid progenies of the cultivar Ruiru 11 was also significant ($p \leq 0.05$) when tested with isolates varying in pathogenicity. Despite the significant main effects of isolates and crosses, their interaction effects were however, non-significant. Estimates of variance components indicated that only a small proportion of variation for resistance was due to genetic effects. The broad sense heritability estimate was only 0.23. Variance due to crosses × isolates interaction was estimated as –0.11, which was interpreted as lack of variance. All isolates were able to induce compatible reactions with varying degrees of aggressiveness among the crosses tested irrespective of the host variety from which they were obtained (Table 3).

Discussion

Pathogenicity tests revealed that out of the 66 Ruiru 11 crosses tested, 50 crosses fell in the medium resistant classes of 7–9 indicating that resistance to CBD in Ruiru 11 is genetically narrow based. It is expected that all the Ruiru 11 crosses carry the T-gene from the Catimor mother parents. Sometimes the T-gene may also be derived from male parents with Hibrido de Timor in their pedigrees. In addition, the male parents may impart the R-gene of resistance if they are derived from crosses with Rume Sudan. However, resistance to CBD carried by some male parents derived from crosses with Rume Sudan and Hibrido de Timor may not be expressed by some Ruiru 11 crosses because the male parents are not genetically fixed for resistance to CBD. This partly explains the variation for resistance, which is observed among crosses with similar pedigrees. The male parents which are useful in imparting good quality and resistance to leaf rust are continuously being selected to obtain types that are true-breeding for CBD resistance. The third recessive k-gene is present in some male parents but is still lacking in the Catimor mother parents. The gene is being introgressed into the Catimor cultivar so that it can eventually be expressed in Ruiru 11 progenies. It is believed that broad-based resistance combining several genes in one plant may not easily break down.

The observation that the main effects of crosses and isolates were significant while their interaction effects were non-significant is an indication that expression of resistance varied with the test isolates and the hybrid crosses but there were hardly any races in the pathogen population. These results are in agreement with the findings of Van der Graaff (1978), Masaba and Van der Vossen (1980) and Omondi et al. (2000) that variation among isolates of *C. kahawae* was predominantly due differences in aggressiveness. In the field, variation may also be attributed to environmental factors, which influence disease epidemics. In the presence of races, the differential effects of cultivar × isolate interaction is expected to be significant. This is manifested by the ability of certain isolates to cause disease on cultivars with compatible resistance genes while non-compatible cultivars remain resistant. In this study, the isolates were aggressive irrespective of the host isolate from which they were obtained. The isolates obtained from Ruiru 11 trees in the field were aggressive on laboratory seedlings of the same cultivar confirming the earlier reports that the hypocotyl inoculation test is highly correlated with field reaction of mature plants to CBD (Van der Vossen et al., 1976). The resistant Ruiru 11 cultivar is grown in the same localities as the susceptible traditional cultivars with high chances of cross infection hence the high ag-

Table 1. The pedigrees and disease scores of Ruiru 11 crosses inoculated with isolates of *C. kahawae*

Male parents (complex crosses)	Catimor mother parents											Male parent means
	CAT.86	CAT.88	CAT.90	CAT.119	CAT.124	CAT.127	CAT.128	CAT.129	CAT.130	CAT132	CAT.134	
SL28 × B3.96 = (RS × SL28)(B × HT)	8.31 B-M	8.11 C-N	–	6.89 L-P	10.28 AB	–	9.48 A-G	8.45 A-L	7.66 E-O	–	9.10 A-J	8.54
SL28 × B3.97 = (RS × SL28)(B × HT)	–	–	–	8.47 A-L	7.95 D-O	–	9.26 A-I	–	–	9.18 A-I	9.97 A-D	8.97
SL28 × B3.99 = (RS × SL28)(B × HT)	10.40 A	6.05 O-Q	6.42 M-Q	–	–	–	9.78 A-D	–	7.31 I-P	–	–	8.00
SL28 × B3.116 = (RS × SL28)(B × HT)	6.97 L-P	7.63 E-Q	8.17 C-M	–	8.28 B-M	8.69 A-L	9.41 A-H	9.07 A-J	8.61 A-L	8.64 A-L	10.04 A-C	8.55
SL28 × B3.185 = (RS × K7)(HT × SL34)	6.95 L-P	6.15 N-Q	–	9.65 A-E	–	9.06 A-J	7.94 D-O	–	–	9.87 A-D	–	8.27
SL28 × B3.863 = (SL34 × RS)HT	7.02 K-P	–	–	7.05 K-P	7.47 G-O	9.00 A-K	10.28 AB	7.41 H-P	8.69 F-O	8.11 C-N	9.01 A-K	8.23
SL28 × B3.866 = (SL34 × RS)HT	6.01 O-Q	–	8.98 A-K	–	7.36 I-P	9.28 A-I	–	8.54 A-L	9.01 A-K	7.31 I-P	7.02 K-P	7.94
SL28 × B3.886 = (SL34 × RS)HT	–	8.69 A-L	6.96 L-P	9.43 A-G	8.39 A-M	–	9.80 A-D	9.49 A-G	9.50 A-F	–	–	8.89
SL28 × B3.887 = (SL34 × RS)HT	8.74 A-L	–	9.23 A-I	–	–	–	–	–	6.45 M-Q	9.40 A-H	–	8.46
SL28 × B3.879 = (SL34 × RS)HT	7.17 J-P	–	–	–	–	–	–	–	–	–	–	7.17
SL28 × B4.691 = (DR1 × HT)(RS × SL28)	–	–	–	–	–	–	4.90 Q	–	–	–	–	4.90
SL28 × B4.609 = (RS × SL28)SL28	5.51 PQ	–	–	–	6.42 M-Q	–	–	–	–	–	–	5.97
Mother parent means	7.45	7.33	7.95	8.30	8.02	9.01	8.85	8.59	8.17	8.75	9.03	8.27

Mean disease scores of crosses followed by the same letter(s) are not significantly different at $p \leq 0.05$ according to Duncan's Multiple Range Test.

Table 2. Analysis of variance on mean grade of infection for hybrid crosses of *C. arabica* cv. 'Ruiru 11' tested with seven isolates of *C. kahawae*

Source of variation	df	Mean square	F-value	Estimated values of components of variance
Isolates	6	180.15**	40.45	$\sigma^2_s = 1.33$
Crosses	65	22.97**	5.16	$\sigma^2_c = 1.32$
Isolates × crosses	390	3.95	0.89	$\sigma^2_{cs} = 0$
Error	462	4.45		$\sigma^2_e = 4.45$

** significant at $p \leq 0.05$.
σ^2_c = variance due to crosses, σ^2_s = variance due to isolates, σ^2_{cs} = variance due to crosses × isolates interactions, σ^2_e = error variance.

Table 3. List of isolates of *C. kahawae* including their sampling locations, host cultivar and disease scores on Ruiru 11 crosses

Isolates	Sampling location	Host cultivar	Disease score on Ruiru 11
R1.6	Gatanga-Thika District (1670 m, 0.92°S, 36.93°E)	Ruiru 11	6.43 E
R2.3	Karatina-Nyeri District (1981 m, 0.49°S, 37.11°E)	Ruiru 11	9.36 A
S2.8	Karatina-Nyeri District (1981 m, 0.49°S, 37.11°E)	SL 28	8.60 B
R3.4	Kiriaini-Muranga District (1760 m, 0.67°S, 36.86°E)	Ruiru 11	7.08 D
R4.5	Kangema-Muranga District (1700 m, 0.68°S, 36.97°E)	Ruiru 11	9.48 A
S9.1	Koru-Kericho District (1615 m, 0.16°S, 35.22°E)	SL 28	7.88 C
S12.8	Jacaranda-Thika District (1603 m, 1.06°S, 36.45°E)	SL 28	8.96 AB

Mean disease score of isolates followed by the same letter(s) are not significantly different at $p \leq 0.05$ according to Duncan's Multiple Range Test

gressiveness of isolates obtained from SL 28 cultivar as well.

Partitioning variance components revealed that only a small proportion of the phenotypic variance for resistance was due to genetic effects. Broad sense heritability was 0.23. This is consistent with the low narrow sense heritability of 0.04 previously estimated in the same Ruiru 11 cultivar using offspring-midparent regression (Omondi, 1994). In contrast, Van der Vossen & Walyaro (1980) observed high heritabilities for resistance to CBD in a genetically heterogenious population. The limited genetic variation among hybrid crosses of the cultivar Ruiru 11 is an indication that the cultivar combines favourable attributes of the two parent populations previously screened and selected for CBD resistance.

Further partitioning of the components of variance revealed that the variance due to isolates × crosses interaction was –0.11. Theoretically, variance estimates cannot be negative. However, the occurrence of negative variance estimates have, been reported several times in literature (ElRouby & Penny, 1967; Leone et al., 1968; Lindsey et al., 1962; Omondi & Ayiecho, 1995.). The negative estimates are usually attributed to some combination of an inadequate genetic model (no epistatic effects in the model), sampling error, inadequate experimental design (competition among individuals) and assortative mating (Lindsey et al., 1962). In this study, the negative estimate of variance was interpreted as lack of variance and no estimation as to the relative importance of each attribute contributing to the occurrence of negative estimates was performed. In conclusion, the Kenyan population of the CBD pathogen is unlikely to have adapted to the cultivar Ruiru 11. It is suggested that pyramiding of resistance genes could create a stable and broad-based resistance that could be effective against pathogen variants.

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