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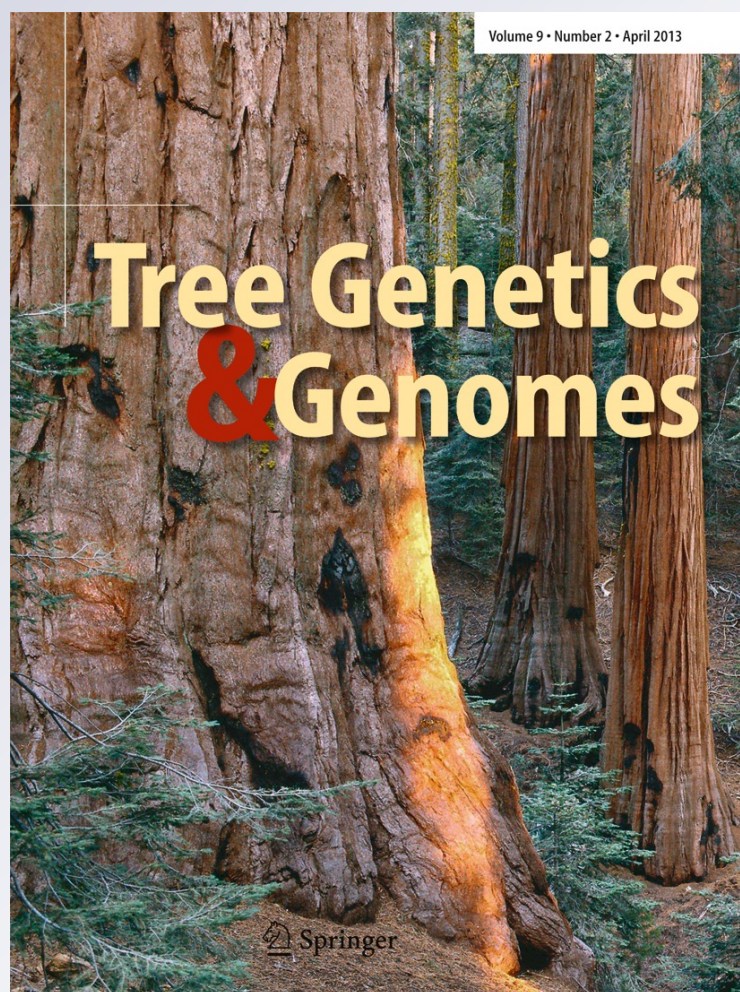
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Inheritance of resistance to coffee wilt disease (*Fusarium xylarioides* Steyaert) in Robusta coffee (*Coffea canephora* Pierre) and breeding perspectives

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Abstract Knowledge on heritability is essential for selecting varieties resistant against coffee wilt disease caused by *Fusarium xylarioides*, which is currently devastating coffee in East and Central Africa. Variability of the resistance against coffee wilt disease in *Coffea canephora* and its inheritance were investigated in three experiments corresponding to a clonal trial involving 20 clones, a 10-parent partial diallel progeny, and a half-sib progeny test. There were significant quantitative genetic variations among the clones and progenies, suggesting polygenic control of the resistance. Around 50–65 % tree mortality was the optimal disease level for calculating heritability and genetic gains. General and specific combining abilities calculated within the optimal disease range for partial diallel analysis were significant. Broad-sense heritability for the same analysis and same disease range was moderate (0.329), and

corresponding narrow-sense heritability was low (0.112). Broad-sense heritability from clones in the field at the same disease range was also moderate (0.333). Narrow-sense heritability from regression of half-sib progeny means onto parent means in the field and screen house was 0.183 and 0.369, respectively. Selecting tolerant clones for improvement against the disease is possible, and genetic gains are possible by using tolerant parents in breeding programs.

Keywords Wilt disease · *Fusarium xylarioides* · *Coffea canephora* · Heritability · Partial resistance

Introduction

Coffee is a very important cash crop especially for about 80 tropical countries, contributing to nearly 7 million tons of green beans produced every year (FAO 2006). Arabica coffee (*Coffea arabica* L.), an inbreeding allotetraploid, and Robusta coffee (*Coffea canephora* Pierre), a self-incompatible diploid, are the main cultivated species among more than 100 species of the genus. In terms of economic importance in international trade, coffee is second to oil, earning more than US \$9,000 million per year (FAO 2006; ICO 2006), and *C. canephora* contributes about 35 % of the world coffee production (ICO 2006). However, this crop is being devastated by coffee wilt disease (CWD) in East and Central Africa. Coffee wilt disease is caused by *Fusarium xylarioides*, a pathogen specific to coffee. Three pathotypes named Robusta, Arabica, and Liberica of this pathogen have been reported (Lepoint et al. 2005). The Robusta pathotype is responsible for the coffee wilt disease on Robusta coffee in Uganda, Tanzania, and the Democratic Republic of

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Congo (DRC) (Flood and Brayford 1997; Oduor et al. 2004). Due to its high potential of spreading, coffee wilt disease is a threat for all the coffee-producing countries.

Coffee wilt is a vascular disease whose symptoms progress from inward curling and wilting of leaves to dieback and death of affected trees. Symptoms may suddenly occur all over the stem, but usually, they start on a single or a few primary branches on one side of the stem and progress laterally until all branches are affected. Symptoms on trees with multistems first appear on a single stem and progress laterally until all stems are affected. The disease kills its host at all ages and within a short period (Musoli et al. 2008). Infested soil may remain infective for several years after uprooting and removing infected trees.

It has become absolutely necessary to control this disease in all affected countries and to prevent it from spreading to new areas. However, currently, there are no effective control measures. Chemical control was found ineffective (Muller 1997), and phytosanitary control measures, which are being emphasized, have been found difficult to implement and ineffective to prevent the disease spread (Wetala et al. 2000). Roguing and burning of affected trees is of some benefit, but adoption is constrained by on-farm resources. Future prospects for the effective management of CWD are thought to depend largely on host resistance, and most research and development efforts are focused in this direction (Rutherford 2006). Prospects for effectively controlling this disease shall depend on replanting with resistant varieties (Delassus 1954), but there are currently no commercial-resistant varieties to be used in the replanting. Resistance against coffee wilt disease has been reported for *C. arabica* (Van der Graaff and Pieters 1978) and *C. canephora* (Fraselle 1950; Meffrein 1957). Frassele (1950) reported resistance to coffee wilt disease among *C. canephora* genotypes/trees under field conditions in the DRC, from which resistant clones were selected and used in the coffee replanting programs within the DRC and Ivory Coast. Resistance has also been observed among progenies of *C. canephora* genotypes from farms and germplasm conservation plots on research stations in Uganda (Musoli 2005), thus offering the opportunity for developing resistant commercial varieties.

In Uganda, breeding for resistance against coffee wilt disease has become the main objective of the *C. canephora* improvement program. Robusta coffee genotypes with variable levels of wilt resistance have been identified and can be used for introgression of resistance into current susceptible commercial clones, which are high yielding, have good quality, and early bearing. However, apart from Van der Graaff and Pieters (1978) mentioning that coffee wilt resistance is polygenic in *C. arabica*, the genetic determinism of the resistance to this disease is still largely unknown. Knowledge on inheritance of the resistance against coffee

wilt disease is vital for designing and implementing an effective program for breeding resistant varieties. We conducted the present study to generate knowledge on genetic control of resistance against coffee wilt disease as a prerequisite to choosing parents and breeding strategies for developing varieties with effective and durable resistance. The specific objectives of the study were: (1) to characterize the inheritance of resistance to coffee wilt disease in Robusta coffee and (2) to propose a strategy for breeding wilt resistant *C. canephora* coffee varieties for Uganda.

Materials and methods

The studies were carried out at Kituza, the headquarters of the Coffee Research Institute (CORI), in Mukono district in Central Uganda, located 25 km East of Kampala (0°21' 12.31" N, 32°45'19.33" E).

Plant material

A three part study was performed:

1. Experiment 1: clonal trial involving 20 clones evaluated in the field.
2. Experiment 2: partial diallel of 35 full-sib progenies multiplied by cuttings and their 10 clonal parents evaluated in the field.
3. Experiment 3: half-sib progeny tested by artificial inoculation of 9–12 months old half-sib progenies of 16 clones in experiment 1 and rooted cuttings of the 11 parents evaluated in the screen house.

In experiment 1, studied clones were raised as rooted nodal cuttings from 20 *C. canephora* genotypes before planting in the field in October 1997 (Table 1). Clones 1 s/2, 1 s/3, 223/32, and 257 s/53 are the commercial clones used also in experiment 2. The rest of the clones in this experiment was selected from progenies of specific crosses involving different parents (Table 1) of which resistance against CWD is not known. Experiment 1 was laid out in a randomized complete block design of four replicates running across the slope, and each replicate divided into 20 plots of six trees arranged in straight line patterns of 3 rows×2 columns, spaced at 3×3 m. Each of the clones was randomly allocated and planted in a plot in each of the replicates. Clones Q/6/1 and Q/1/1 were only planted in replicates 1 and 2 due to insufficient planting materials. Their plots in replicates 3 and 4 remained without plants. Clones H/4/1 and R/14 were not planted in replicate 4 because of a similar reason. The experiment was surrounded by two guard lines of nonexperimental materials and a guard line in between each plot.

In experiment 2, full-sib progenies studied were generated from a 10-parent diallel cross (Table 2) and were

Table 1 *C. canephora* clones studied in experiment 1 and their rooted cuttings and half-sib progenies in experiment 3

Clone	Type of source material	Source collect/progeny	Experiment 1	Experiment 3	
			No. of plants	No. of cuttings	No. of seedlings
1 s/2	Open pollinated ^a	Farm collections	24	12	20
1 s/3	Open pollinated ^a	Farm collections	24		20
223/32	Open pollinated ^a	Farm collections	24	10	20
257/53	Open pollinated ^a	Farm collections	24	20	20
B/1/1	Specific hybrid	1 s/6×4/6	24		20
B/2/1	Specific hybrid	1 s/6×4/6	24	7	
B/6/2	Specific hybrid	1 s/6×4/6	24		19
C/1/7	Specific hybrid	1 s/6×245/62	24	11	20
C/6/1	Specific hybrid	1 s/6×245/62	24		20
E/3/2	Specific hybrid	1 s/2×245/62	24		20
G/3/7	Specific hybrid	227/61×1 s/2	24	20	20
H/4/1	Specific hybrid	257/18×1 s/2	18	20	20
J/1/1	Specific hybrid	227/61×1 s/6	24	20	
L/2/7	Specific hybrid	4/6×227/61	24	12	20
P/3/6	Specific hybrid	218/32×1 s/6	24		16
P/5/1	Specific hybrid	218/32×1 s/6	24		
Q/1/1	Specific hybrid	224/64×1 s/6	12		17
Q/3/4	Specific hybrid	224/64×1 s/6	24	20	
Q/6/1	Specific hybrid	224/64×1 s/6	12		20
R/1/4	Specific hybrid	224/64×245/62	18	9	20

^a Commercial variety

analyzed as partial diallel with bulked reciprocals, considering that maternal effects are low for many traits in *C. canephora* (Montagnon 2000). The parents 1 s/2, 1 s/3, 1 s/6, 223/32, 257 s/53, and 258 s/24 are current commercial clones. Clones 259 s/56 and 236/26 were dropped from the commercial list due to their susceptibility to leaf rust (*Hemileia vastatrix*). Parent 245/62 only exists in the conservation plots at CORI. All of these clones are descendants of single-tree selections among open-pollinated individuals collected from farms in

different parts of Uganda in the 1930s–1950s (Thomas 1947; Leakey 1970). Parent J1/14 is a single-tree selection among open-pollinated progenies introduced from Indonesia in the 1930s. The parents were selected without prior knowledge about their resistance against the coffee wilt disease. Seedlings of the 35 progenies studied were raised in the coffee nursery before planting out in the field. Rooted cuttings of the 10 parents were also raised for inclusion in the experiment. This experiment was planted in May 1997. The experiment

Table 2 *C. canephora* diallel cross progenies studied in experiment 2 and number of genotypes per progeny

Parents	1 s/2	1 s/3	1 s/6	223/32	257 s/53	258 s/24	236/26	259 s/56	245/65	J1/14
1 s/2		12		12	12					
1 s/3				12				12		12
1 s/6		12		12	12			12		12
223/32			12			12				
257 s/53										
258 s/24			12	12				12	12	
236/26	12							12	12	12
259/56	12				12	12			12	
245/62	12	12	12	12		12				
J1/14	12	12				12		12	12	

was laid out in a randomized complete block design with two replicates running across the slope, and each replicate was divided into 45 plots of six trees arranged in single straight-line pattern at a spacing of 3×3 m. Each of the hybrid progenies and parent clones were randomly allocated and planted in plots in each of the replicates. Two guard lines of mixed open-pollinated seedlings of nonexperimental materials were planted all round the experiment and between replicates.

Both experiments 1 and 2 were weeded, trained, desuckered, and supplied with organic and inorganic fertilizers following routine procedures of maintaining *C. canephora* gardens in Uganda. In experiment 3, 16 half-sib progenies were raised from open-pollinated seeds of 16 *C. canephora* clones studied in experiment 1 (Table 1). In addition, rooted nodal cuttings from 11 out of the 20 clones studied in experiment 1 were also analyzed in experiment 3 (Table 1). The seedlings and rooted clonal cuttings were raised in the coffee nursery at CORI following routine procedures.

Infection of plant materials

Plants in experiments 1 and 2 (fields) were naturally infected with *F. xylarioides* inoculum available in the fields. Plants studied in experiment 3 (screen house) were artificially inoculated with *F. xylarioides* by root dipping. At 9–12 months old, the plants were stripped off all planting medium (soil), and their roots were washed under tap water before dipping the entire root section for at least 30 min into a water suspension inoculum at 1.0×10^6 spores/ml. The inoculum was prepared from *F. xylarioides* single-spore isolate derived from infected stem parts of Robusta coffee clone 257/53. After inoculation, the plants were replanted in polythene pots filled with fresh soil rich in organic nutrients and kept in the screen house for incubation at unregulated room conditions. The plants were regularly watered with tap water using a watering can and monitored for the disease symptoms.

Data collection

Coffee plants for experiments 1 and 2 were planted in 1997. At this time, coffee wilt disease did not exist at CORI. In 1999, the disease started affecting coffee plants in the two experiments. Assessing for resistance against coffee wilt disease began in experiment 1 in April 2001. Assessments started in experiment 2 in December 2002 when coffee plants were 5 years old. For both experiments, incidence of the disease (D'Arcy et al. 2001) was measured by percent defoliation of trees. A plant with 75 to 100 % of defoliation is considered as dead. Data were recorded twice every month from time of beginning up to March 2006 to allow assessment of disease enhancement in time and space. A

total of 81 data sets were collected on 81 assessment dates for experiment 2, and 130 records were collected for experiment 1. Nonexperimental coffee trees in rows and columns surrounding experiments and separating replicates were not assessed.

Plants in experiment 3 were assessed for response to coffee wilt infection every week starting from week 5 after inoculation up to week 10. The plants were assessed on percentage of mortality.

Data analysis

Analysis of phenotypic variations

Analysis of variance (ANOVA) was performed on the percentage of mortality data using the general linear model of the Statistical Analysis System (SAS) program (SAS Institute Inc., Cary, NC; SAS 1989) to determine statistical significance of observed variations. For experiments 1 and 2, the ANOVA was performed on disease incidence data at monthly intervals including the first and last assessments. The model used for the three experiments is the following:

$$Y = \mu + \text{replicate}_i + \text{clone}_j \left(\text{or family}_j \right) + e_{ijk}$$

where replicate_{*i*} is the effect of replication *i*; clone_{*j*} (or family_{*j*}) is the effect of clone (or family) *j*, and *e_{ijk}*, the residual term. All the effects are considered as fixed. The three experiments were analyzed with the same model separately.

For experiment 3, the ANOVA was performed on disease incidence data at 10 weeks postinoculation. The progenies and parents in experiments 1 and 3 were analyzed independently. Student-Newman-Keuls mean separation tests were performed to rank means for all experimental data sets where ANOVA was significant. Percent tree mortality was computed for all data sets where ANOVA was performed. The progeny and/or clone means in the various experiments were grouped into classes based on the percentage of mortality of 0 % which is resistant; 1 to 15 % is moderately resistant; 16 to 50 % is moderately susceptible; 51 to 75 % is susceptible, and >75 % is highly susceptible.

Analysis of genetic variations

Components of genetic variance, narrow (h_n^2) and broad (h_b^2) sense heritabilities, were calculated for disease incidence data of the first assessment date and dates after every 6 months up to December 2005 plus March 2006 for the partial diallel progenies in experiment 2. The calculations were performed with Diogene quantitative genetics software (Baradat and Labé 1995) using Garretsen and Keuls random

model (Keuls and Garretsen 1977). Linear model assumptions were checked for each trait. When necessary, data were adjusted for the block effect, prior to the decomposition of the family effect (partial diallel analysis). The analysis of the partial diallel was carried out using the random model:

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the value of the trait for individual k corresponding to the cross between parents i and j ; g_i is the general combining ability (GCA) of i th parent, and g_j is the general combining ability of the j th parent; s_{ij} is the specific combining ability (SCA) of the cross between i th parent and j th parent. Broad-sense heritability was calculated as: $h_b^2 = V_g/V_p$, where V_g is the genotypic variance, and V_p is the phenotypic variance component. Narrow-sense heritability (h_n^2) was calculated as: $h_n^2 = V_a/V_p$, where V_a is the additive variance component. The confidence intervals at 95 % for these estimations were obtained by the Jackknife method (Shao and Tu 1995).

Broad-sense heritability values were also estimated from experiment 1. These values, along with associated confidence intervals, were calculated from data of the first assessment date (April 2001) and dates after every month until March 2006, using SAS software and the Wald model (Agresti and Coull 1998).

Two values of narrow-sense heritability were estimated from the screen house studies: first, as twice the slope of the regression of means of the open-pollinated progenies (half sibs) in experiment 3 onto the means of their parent clones within the same experiment; and second, as twice the slope of the regression of the progeny means onto the means of their parent clones in field experiment 1 (Falconer 1996). The means of the last assessment dates (week 10 postinoculation for screen house experiment 3 and March 2006 for field experiment 2) were used in the regression. The regression was performed using the SAS software.

It should be noted that progenies from the most resistant clones J/1/1 and Q/3/4 were inadvertently not included in this analysis, and there was no prior knowledge about the relationship of progeny response to infection in the screen house and the response of their clonal parents either within the screen house or in the field.

Estimation of expected genetic gains

Expected genetic gains from selecting clones in experiment 1 were calculated for analyzed data sets as: $\%gg = 100 ((M_c - M_r)/M_c)$, where $\%gg$ is genetic gain; M_c is mean mortality of current commercial clones; M_r is mean mortality of selected resistant clones.

Results

Phenotypic variations

In experiment 1, analysis of variance revealed highly significant ($p < 0.001$) differences between the clones for all percentage of mortality data sets analyzed (Table 4, ANOVA of first and last assessments as example). Differences between replicates in experiment 1 were not significant for all the data sets. Student-Newman-Keuls mean separation tests grouped means into overlapping classes that varied from four to seven, depending on the assessment date (data not shown). Diseased trees die within 1 to 15 months, but often as short as 6 months. The dynamic of the disease is different with the clones (Fig. 1). When assessment started in April 2001 in experiment 1, the mortality varied from 0 % (no disease) observed on clone J/1/1 to 62 % on clone P/3/6. In March 2006 (5th year of assessment), average mortality on the most susceptible clones P/3/6, H/4/1, E/3/2, and B/6/2 varied from 87 to 96 %. Clone J/1/1 did not succumb to the disease in this experiment. The overall mean for this date was 50 % of dead plant. When the clone means for the last assessment were grouped based on the resistance scale, 45 % of the clones were highly susceptible; 25 % were susceptible; 20 % were moderately susceptible; 5 % were moderately resistant, and 5 % were resistant (Table 5). Only clone J/1/1 was in the resistant category, and clone Q/3/4 was in the moderately resistant class. Clones C/1/7, B/6/2, H/4/1, and P/3/6 were among the highly susceptible clones. The results showed highly significant genetic differences between clones with two clearly resistant clones J/1/1 and Q/3/4.

In experiment 2, analysis of variance found significant differences for the percentage of mortality on the parents and progenies (Table 3). Student-Newman-Keuls mean separation test ranked the parent means into three overlapping classes a, b, and c for all data sets where ANOVA was performed (data not shown). The mortality among parents at the beginning of the assessment varied from 17 %

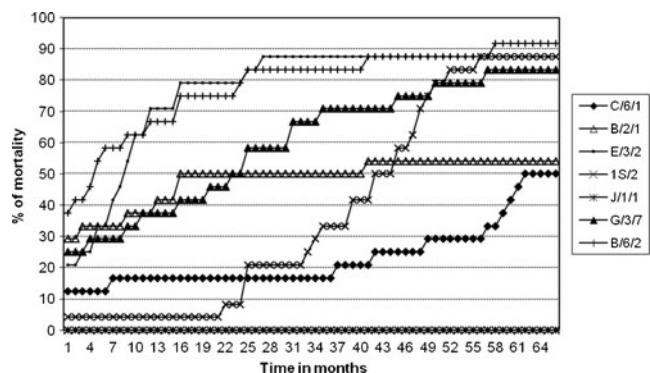


Fig. 1 Progression of plant mortality among *C. canephora* clones (experiment 1)

Table 3 Analysis of variance for coffee wilt disease incidence on progenies and parents in experiment 2

Analysis	Assessment date	Assessment record	Source	DF	SS	F value	Pr>F	CV (%)	Mean disease level
Parents	4 Dec 2002	1 (first)	Parents	9	154.7	5.98	<0.0001	54.09	3.1
		1 (first)	Replicate	1	4.03	1.4	0.238 NS		
	31 Mar 2006	41 (last)	Parents	9	88.8	3.52	<0.0001	43.83	3.8
		41 (last)	Replicate	1	0.03	0.01	0.913 NS		
Progenies	4 Dec 2002	1 (first)	Family	34	300.39	2.73	<0.0001	53.31	3.4
		1 (first)	Replicate	1	4.03	1.4	0.238 NS		
	31 Mar 2006	41 (last)	Family	34	128.56	1.62	0.017 NS	36.29	4.2
		41 (last)	Replicate	1	0.77	0.33	0.566 NS		

CV coefficient of phenotypic variation, DF degree of freedom, SS sum of squares, Pr probability, NS not significant

observed on parents 1 s/2 and 1 s/3 to 92 and 83 % observed on parents 257 s/53 and J1/14, respectively, with overall mean of 52 %. By the last assessment, the mean mortality among the parents varied from 33 % observed on parent 236/26, the least susceptible, to 100 % on parent 257 s/53, with overall mean of 71 %. When parent means for the last assessment were grouped into classes, 50 % of them were highly susceptible; 30 % were susceptible, and 20 % were moderately susceptible (Table 5).

Student-Newman-Keuls mean separation test ranked the progeny means into three overlapping classes for assessments in December 2002–April 2003 (data not shown). Progeny means for assessments after this date were ranked in two overlapping classes. The mortality among progenies at the beginning of the assessment varied from 17 % on 245/62×258 s/24 to 92 % on J1/14×1 s/3, with overall mean of 57 %. By the last assessment in March 2006, the mortality varied from 42 % observed on progeny 236/26×1 s/2, the least susceptible, to 100 % on progenies 259 s/56×1 s/2, J1/14×259 s/56, 1 s/6×J1/14, J1/14×1 s/3, and J1/14×258 s/24. The overall mean at this date was 80 %. Grouping of progeny means for the last assessment shows that 60 % of them were highly susceptible; 37 % were susceptible, and only 3 % were moderately susceptible. There were no progenies in the moderately resistant and resistant classes

(Table 5). These results showed significant genetic differences for CWD resistance among parent clones and their progenies, but the overall level of resistance for both remained low.

In experiment 3, analysis of variance performed on the percentage of mortality data of the clones, at week 10 after inoculation, found highly significant ($p<0.001$) differences between clones (Table 4). Student-Newman-Keuls mean separation test showed three overlapping classes a, b, and c (data not shown). At first assessment, average percentage mortality varied among clones from 0 % (no disease) on clones 257/53 and 1 s/2 to 4 % on cuttings of clone R/1/4. By week 10 after inoculation, mortality on the most susceptible clones 1 s/2, 223/32, G/3/7, and H/4/1 was 100 % (all plants dead), 90, 80, and 70 %, respectively, and on the least affected clones J/1/1 and B/2/1, 15 and 0 % of dead plants, respectively. When the clone means of assessment at week 10 were grouped into resistance scale, 18 % of the clones were highly susceptible; 27 % were susceptible, and 36 % were moderately susceptible. J1/1 is highly resistant, and B/2/1 is the only clone resistant (Table 5).

The screen house results confirm that clones in field experiment 1 have varying levels of resistance to coffee wilt disease, and the genetic differences between the clones are highly significant. Clones J/1/1 and Q/3/4, which were

Table 4 Analysis of variance for coffee wilt disease incidence on clones in experiments 1 and 3 and their progenies in experiment 3

Experiment	Assessment date	Assessment record	Source	DF	F value	Pr>F	CV	Mean mortality (%)
1	27 Apr 2001	1 (first)	Clone	19	4.89	<0.0001	76.1	25.3
1	27 Apr 2001	1 (first)	Replicate	3	0.24	0.87 NS	76.1	
1	31 Mar 2006	130 (last)	Clone	19	13.01	<0.0001	41.82	64.5
1	31 Mar 2006	130 (last)	Replicate	3	1.8	0.15 NS	41.82	
3	12 Jun 2006	6 (week 10)	Clone	10	7.76	<0.0001	48.4	50.7
3	12 Jun 2006	6 (week 10)	Progeny	15	5.37	<0.0001	44.9	51.8

Family in experiment 1 includes both progenies and parent clones

CV coefficient of phenotypic variation, DF degree of freedom, Pr probability, NS not significant

Table 5 Distribution of progenies and clones in experiments 1 to 3 on the 1–5 disease incidence scale

Experiment	Individuals (total)	No. of progenies or clones	Resistance scale (% dead plant)				
			R 0	MR 1–15	MS 16–50	S 51–80	HS (>80)
2 (parents)	120	10	0	0	2	3	5
2 (full-sib progenies)	420	35	0	0	1	13	21
1 (mature clones)	444	20	1	1	4	5	9
3 (young clones)	161	11	1	1	4	3	2
3 (half-sib progenies)	312	16	0	1	4	7	3

R resistant, MR moderately resistant, MS moderately susceptible, S susceptible, HS highly susceptible

respectively rated as resistant and moderately resistant in the field, succumbed to CWD in the screen house but were still ranked most resistant.

Analysis of variance performed on the disease incidence data on half-sib progenies at week 10 after inoculation found highly significant ($p < 0.0001$) differences between progeny means (Table 4). Student-Newman-Keuls mean separation test showed three overlapping classes a, b, and c (data not shown). Ten weeks after inoculation, percentage of mortality on the most susceptible clones C/1/7, B/1/1, and Q/6/1 was 95 % of dead plants, 80, and 85 %, respectively, and incidence on least affected clones B/2/1 was 10 %. When the progeny means of the assessment at week 10 were grouped into resistance classes, 63 % of them belong to highly susceptible and susceptible groups. No progeny was in resistant classes (Table 5). These results also showed significant genetic differences between progenies with a low level of resistance.

Decomposition of genetic variances and estimation of genetic control

Broad-sense heritability estimates from clones in experiment 1 were moderate, varying from 0.148 observed in April 2001 (first assessment) to 0.355 observed in November and December 2005 (maximum heritability) (Table 7). The broad-sense heritability estimates increased with subsequent assessment until November and December 2005, when maximum values were obtained, before decreasing to 0.350 in February 2006 and to 0.347 in March 2006.

Estimates of variance components (dominance and additive) and associated specific and general combining abilities for experiment 2 are presented in Table 6. Broad- and narrow-sense heritability values and their confidence intervals are given in Table 6 for the first assessment and successive assessments every 6 months until March 2006. The general combining ability was significant for all data sets.

Table 6 Variance components, heritability estimates, and disease incidence in experiment 2

Date	V_p	V_g	V_d	V_a	V_e	SCA	GCA	h^2_b	h^2_n	Tree mortality (%)
Dec 2002	57.35	19.04	12.6	6.44	19.27	1.841 ^a	4.199 ^b	0.329 (0.041–0.618)	0.112 (0.000–0.249)	56.7
Jun 2003	51.13	13.65	7.66	6.00	23.82	1.564 NS	3.964 ^b	0.264 (0.000–0.529)	0.117 (0.000–0.247)	65.7
Dec 2003	44.31	9.79	4.74	5.05	24.73	1.399 NS	3.695 ^c	0.219 (0.000–0.481)	0.114 (0.000–0.235)	71.2
Jun 2004	43.04	7.06	2.26	4.80	28.92	1.192 NS	3.388 ^c	0.162 (0.000–0.409)	0.111 (0.000–0.227)	73.8
Dec 2004	40.76	5.10	0.57	4.52	30.57	1.051 NS	3.192 ^c	0.123 (0.000–0.362)	0.110 (0.000–0.228)	76.9
Jun 2005	39.98	4.40	0.00	4.40	31.18	0.978 NS	3.093 ^d	0.110 (0.000–0.236)	0.110 (0.000–0.223)	78.3
Nov 2005	38.65	3.98	0.00	3.98	30.69	1.549 NS	2.822 ^d	0.102 (0.000–0.213)	0.102 (0.000–0.213)	79.8
Mar 2006	37.5	3.41	0.00	3.41	30.68	0.90 NS	2.618 ^d	0.090 (0.000–0.196)	0.090 (0.000–0.196)	77.9

V_d variance due to dominance, V_a additive variance (breeding value), V_g total genotypic variance, V_p phenotypic variance, V_e environmental variance, SCA specific combining ability, GCA general combining ability, h^2_n narrow-sense heritability, h^2_b broad-sense heritability

^a Significant at 5 % probability

^b Significant at 0.01 %

^c Significant at 0.1 %

^d Significant at 1 %

This implies that observed variability between crosses for disease incidence due to additive interactions was significant and influenced the heritability estimates. Corresponding specific combining ability was significant at 5 % for only the first assessment (December 2002), implying that variability between crosses due to interaction of additive effects among parents is significant for this date. Generally, additive and dominance variances calculated for these data sets were low compared to the environmental variance (Table 6). The genetic variance components drastically decreased, corresponding to increasing tree mortality. Resulting broad (h_b^2) and narrow (h_n^2) sense heritability estimates drastically reduced with successive assessments. The broad-sense heritability (h_b^2) varied from moderate (0.329) observed for the first assessments and continuously decreased to as low as 0.091 observed for the last assessment. Concomitantly, the narrow-sense heritability values remained almost stable at the different dates of assessment from 0.112 to 0.091. Corresponding confidence intervals were large, and they included zero, except for the first assessment, confirming the low precisions generally observed for heritability estimates (Falconer 1996) when samples size is low. Within the disease range of 55–65 %, the broad-sense heritability estimates from experiments 1 and 2 are comparable (Table 6). This threshold has been found convenient for assessing partial resistance of black pod disease in cocoa caused by *Phytophthora megakarya* (Berry and Cilas 1994).

In experiment 3, narrow-sense heritability values estimated by regressing disease means of the last assessment for half-sib progenies in the screen house onto means of the last assessment for their clonal parents in the field (experiment 2) and parents within the screen house (experiment 3) were 0.183 and 0.369, respectively. Both estimates are comparable to estimates in field experiments 1 and 2. However, the

coefficients were not significant, implying that the values are not different from zero.

Expected genetic gains

Expected genetic gains calculated from experiment 1 are given in Table 7. It should be noted that gains of selecting the resistant clones J/1/1 and Q/3/4 in experiment 1 against the mean performance of current commercial clones is very high (96.8 %). These high genetic gains relied on the fact that the two clones J/1/1 and Q/3/4 are resistant, as such clonal selection of these genotypes will obviously be extremely valuable.

Discussion

Few studies have reported host resistance for coffee wilt disease (Meffrein 1957; Delassus 1954), but no study has reported the host genetic systems that control this resistance. The objective of this study was to analyze the CWD resistance and to evaluate its heritabilities.

The field resistance of *C. canephora* (exp 1) showed a percentage of mortality from 0 to 96 %, with clones J/1/1 and Q/3/4 resistant and highly resistant; the disease period ranging from 1 to 16 months was also very variable between the clones. These results suggest a variation in resistance mechanism between the clones.

Although significant genetic variations were detected between both parents and progenies in experiment 2, all the parents and their progenies were susceptible to coffee wilt disease. This implies that the variability of phenotypic variances was small, since over 97 % of the progenies were within the range of 3–5 on the disease incidence scale.

Table 7 Heritability and genetic gains estimates in experiment 1 and corresponding tree mortality and disease incidence

Date	Confidence interval (h_b^2)	Disease incidence (1–5 scale)	Percent genetic gains	Percent tree mortality
Apr 2001	0.148 (0.045–0.252)	1.13	100	25.3
Jun 2001	0.180 (0.064–0.295)	1.31	100	28.1
Dec 2001	0.241 (0.106–0.377)	1.82	100	38.0
Jun 2002	0.274 (0.129–0.418)	2.04	100	43.7
Dec 2002	0.295 (0.146–0.444)	2.33	100	50.0
Jun 2003	0.312 (0.160–0.465)	2.42	94.3	50.6
Dec 2003	0.331 (0.174–0.487)	2.72	94.7	53.2
Jun 2004	0.333 (0.176–0.490)	2.83	95.7	56.8
Dec 2004	0.349 (0.189–0.508)	2.99	96.4	60.2
Jun 2005	0.354 (0.194–0.515)	3.08	96.8	62.2
Nov 2005	0.355 (0.194–0.515)	3.16	96.8	64.5
Mar 2006	0.347 (0.188–0.506)	3.18	96.8	64.5

Phenotypic variations between clones used in experiment 3 were much more significant than in exp 1. Clones J/1/1 and Q/3/4, which were respectively resistant and highly resistant in the experiment 1, succumbed to infection in the screen house experiment, where clone J/1/1 was classified as resistant, and Q/3/4, as moderately susceptible; however, they remained as the most resistant. This demonstrated that screen house and field results are complementary, with different levels of inoculum.

This study detected significant genetic differences among coffee clones and progenies and allowed estimation of genetic parameters for predicting gains of selecting and breeding against coffee wilt disease. Quantitative variations between progenies and/or clones for disease incidence in all the experiments suggest that resistance against coffee wilt disease in Robusta is governed by many genes, which are variably distributed among genotypes. The most resistant genotypes J/1/1 and Q/3/4 are presumed to have the largest number of resistance genes (Agrios 1997). This concurs with results of resistance tests performed on open-pollinated progenies of individuals from different sources (Kalangala islands, Itwara and Kibale primary forests, and Nganda and Erect phenotypes), conducted in an experiment outside the current study, which also showed that the resistance genes/factors are variably distributed among *C. canephora* populations (Musoli, unpublished data). Quantitative or polygenic resistance or partial resistance/horizontal is considered durable (Van der Plank 1963), and it has been reported for many crop plant diseases (Milliano et al. 1986; Lespinasse et al. 2000; Zahirul et al. 2005; Le Guen et al. 2007; Colon et al. 1995). Among coffee diseases, polygenic resistance has been reported for coffee berry disease caused by *Colletotricum kahawae* in *C. arabica* (Van der Graaff 1978).

In this study, disease level of 55–65 % tree mortality was considered logical for contrasts, since expression of the resistance traits is explicit when there is sufficient disease in the field (Berry and Cilas 1994), as determined from the level of infection on susceptible genotypes. Disease levels below this range would be inappropriate since it takes time for natural field infections to have sufficient inoculum to reach all trees (Zadoks and Schein 2003). Higher disease levels would also be inappropriate as moderately resistant genotypes can also develop the disease at high inoculum pressure. Incomplete polygenic resistance is valuable for its delaying the development of the disease.

Although heritability and expected genetic gains were estimated for wider range of disease levels, we consider values estimated within the disease range of 50–65 % tree mortality to be more meaningful for effective breeding against coffee wilt disease. More of our highest heritability values were obtained within this disease range.

Narrow-sense heritability value derived from regression of half-sib means onto means of their clonal parents in the

screen house (0.369) was higher than heritability estimated from regression of the progeny means onto parental means in the field (0.183). This is certainly expected since the screen house experiments are closely related in a way that plant materials used in both experiments were of the same age, and infection protocol and care for the material during experiment period was the same. The former value is comparable to estimates derived from the clones in the field within the optimal disease range.

It seems that it is the first study on heritability of disease resistance in Robusta coffee, but low to moderate narrow- and broad-sense heritabilities have been reported for other quantitative traits in this crop (Leroy et al. 1994; Cilas et al. 2003, 2011; Montagnon et al. 2003). It would be interesting to study the correlations between resistance to coffee wilt disease and these other traits. Low to moderate heritability has been reported for partial resistance in some other crops (Capettini et al. 2003), and for most of them, it has been indicated that developing resistant varieties through breeding is difficult.

Because of low narrow-sense heritability calculated from experiment 2 and the small associated genetic gain (2–3 %), it would be difficult to derive hybrid populations resistant against coffee wilt disease using parents from this experiment. It should be noted that the most resistant clones J/1/1 and Q/3/4 were not included in this trial because there was no information available on them; neither can we select any of the parents as a source of resistance for a breeding program or clone for farmer adoption. The moderate broad-sense heritability observed for genotypes in experiments 1 and 3 and the wide phenotypic variation observed among genotypes in these experiments demonstrate potential for selecting wilt-resistant clones from the *C. canephora* populations. The theoretical gains in calculations based on selecting resistant clones J/1/1 and Q/3/4 in experiment 1 are clearly high, which can be made available for large-scale vegetative propagation through nodal stem cuttings or somatic embryogenesis. Large-scale germplasm screening may be carried out to identify more clones to supplement J/1/1 and Q/3/4. In the long term, introgression of resistance from clones J/1/1 and Q/3/4 into the current commercial and associated effects on other agronomic and quality traits should be considered.

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