

Expected selection efficiency for resistance to cacao pod rot (*Phytophthora palmivora*) comparing leaf disc inoculations with field observations

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Summary

A genetic analysis of resistance of cacao to *Phytophthora palmivora* was carried out in a 5 × 5 diallel and in a 4 × 2 NC II factorial design, involving cross-progenies and parental clones planted in the nursery and field, respectively. Resistance was scored in the laboratory by inoculation of leaf discs with *P. palmivora* spores with four replicates and, for the factorial design, results were compared with the percentage of rotten pods in the field assessed over a seven-year period. Significant differences between genotypes were observed for both laboratory and field evaluation methods. For the laboratory test, no reciprocal effect was observed and plant effects within seedling progenies were more significant than plant effects within clones. General combining ability was the main source of variation but specific combining ability was also significant for the leaf disc test applied to the diallel. Heritabilities increased with the number of replicates, reaching values of 0.34 and 0.67 for narrow sense heritability, and 0.60 and 0.67 for broad sense heritability, for the diallel and the factorial design, respectively. These values were higher than observed for the percentage of rotten pods in the field in the factorial design (0.42 and 0.47, respectively). For the leaf test, the expected genetic gains were around 30% with a selection intensity of 5%. The existence of significant genetic (0.71) and phenotypic (0.39) coefficients of correlation between resistance on leaves and percentage of rotten pods in the field in the factorial design confirms the feasibility of using the leaf disc test for early selection of resistance to *Phytophthora* pod rot of cacao.

Introduction

Black pod, or *Phytophthora* pod rot, is the most common disease of the cacao tree worldwide, particularly on the African continent, which supplies 80% of world cocoa production. The most aggressive species of *Phytophthora* in the field is *P. megakarya*, causing losses of up to 80% in areas affected by this fungus (Cameroon, Nigeria, part of Ghana, and near to the Ghanaian border in Côte d'Ivoire). In the next 10–15 years, this disease could spread to all the cacao growing zones in Ghana and Côte d'Ivoire. The likely outcome is a 20 to 40% increase in pod losses due to *Phytophthora* in these countries, which supply together about 60% of world cocoa production.

Although expensive and constraining for the environment, application of chemical fungicides still

remains largely the only method to control this disease in the most seriously affected countries. However, it has already been shown that genetic resistance is a promising way of fighting the disease. Genetic parameters of resistance to the disease were studied in several countries, in order to more effectively guide the selection of resistance to black pod (e.g. Cilas et al., 1998; Rodriguez et al., 1985; Tan & Tan, 1990). Different ways of assessing resistance were tested (Blaha, 1974), mainly by observing infection levels in the field and by carrying out artificial inoculation tests on attached or detached pods. One of the major drawbacks of these methods lays in the long time lapse between new crosses and evaluation of resistance (4 to 5 years for the pod inoculation test and 8–10 years for observations on natural infection in the field). Assessing resistance in an early stage, using organs other than pods, is

now a priority for most breeding programmes (Nyassé et al., 1995).

In this study, resistance to *P. palmivora* was assessed using inoculations of leaf discs, which would enable early evaluation. The data obtained were used to estimate heritability and genetic gain that might be obtained by early selection for resistance to the pathogen. For this, the leaf disc test was applied to two mating designs, a 5×5 diallel (using young nursery plants), and a 4×2 factorial design (using mature field plants). The results of the leaf disc test for the factorial design were compared with the level of field infection of the same progenies.

Material and methods

Plant material

The seedlings used in the nursery belong to a 5×5 diallel mating design involving ten crosses and five selfings, each of 30 seedlings of 5 to 8-months old. The parents involved were known for their yield potential and for their variable levels of susceptibility to *P. palmivora*. These were: PA150 (resistant), P7 (resistant), T60/887 (moderately resistant), "H" (progeny selected from the cross UPA402 \times UF676, moderately resistant) and IFC1 (susceptible). The progenies were deployed in three blocks in the nursery, each containing 10 plants per progeny. The parental clones, each represented by five rooted cuttings, were placed nearby the seedling progenies.

The trees in the field are part of a 4×2 factorial mating design, planted at the research station of CNRA in Bingerville, Côte d'Ivoire. Each of the 8 progenies comprised 30 trees that were 11 years old at the time of the leaf disc inoculations. The field design was totally randomized single trees, planted in three adjacent blocks at a density of 1333 trees per hectare. The female parental clones (PA13, P19A, PA121 and IS89) and male parental clones (IMC67 and PA150) were each represented by five adult trees planted in rows in a small germplasm collection, which was nearby the factorial progeny trial. Three trees per parent were used for the leaf disc inoculation tests.

Fungal material

An isolate of *P. palmivora*, the predominant species in Côte d'Ivoire, was used to prepare the inoculum (zoospore suspension) for artificial inoculations on

leaves. This isolate was collected from a pod that was naturally infected with *P. palmivora* collected in a progeny trial set up at Bingerville in 1988 and maintained in the laboratory since, with regular passages through inoculation in pod tissue to maintain aggressiveness of the isolate (Tahi et al., 2000).

Leaf sampling and preparation

For each of the inoculations series, leaves were collected in 2 or 3 batches representing all the parents and all the progenies of each mating design. The batches were made up of 15 different plants per progeny in the nursery and of 10 different plants per progeny in the field. The parents were represented in each batch by five rooted cuttings in the case of the diallel mating design and by three trees for the factorial design. These were used as controls to make the link between the different batches. All together, the nursery batches contained 250 plants inoculated together in one series, and the field batches 98 plants each. In the nursery, two leaves were taken per plant between 6:30 and 9:00 am from semi-lignified seedling stems (for the nursery progenies) and from semi-lignified plagiotropic twigs (for the parental control clones). In the field, five leaves were harvested from semi-lignified plagiotropic twigs growing in medium-shaded zones in the canopies between 6:30 am and 2:00 pm.

After collection, each of the leaves was placed in numbered plastic bags in which a few drops of distilled water were sprayed beforehand. The bags were then kept in the dark till the next morning, when leaf discs were prepared. This was done to minimise any effect of leaf sampling time that may occur with large time lapses between harvesting of leaves (Tahi, 2003). In all, four test series (replicates) were conducted for each mating design, with 14 to 30 days intervals between two consecutive series, during the rainy season (between April and November).

Preparation of leaf discs and inoculation method

Leaf discs of 15 mm in diameter were cut with a cork borer. In total, 12 discs were taken per leaf from the nursery plants and 8 discs per leaf from the mature trees in the field. All the discs from the same plant were mixed prior to being placed in four trays of $70 \times 60 \times 10$ cm on wetted plastic foam of one cm thick and imbibed with 2.5 L of distilled water per tray. The discs from the same plant were aligned in totally

randomized rows of six discs per tray for the nursery plants and of ten discs per tray for the mature trees.

The inoculation method used was based on that of Nyassé et al. (1995). After calibration to 3.10^5 zoospores per mL, all the discs were inoculated by depositing a 10 μ L drop of spore suspension in the middle of each disc. The discs of each plant were inoculated transversally to the rows, so as to randomise any effect of the spore batch equally over the different genotypes.

Observation of symptoms

Symptoms were scored 7 days after inoculation using a 0 to 5 point scale depending on the size of necrosis (0 = no symptoms, 5 = appearance of a large spreading necrotic lesions), as described by Nyassé et al. (1995).

Natural pod infection in the field has been evaluated from 1990 to 1997 on individual trees of the factorial mating design during the harvesting periods. Pods attacked by *Phytophthora* (Bp), rodent damaged pods (R) and healthy ripe pods (H) were counted each month, with elimination of completely rotten pods after each harvesting round. The percentages (%) of rotten pods (Bp) were estimated in relation to the total number of pods produced by the tree: $\% \text{ Bp} = 100 \times \text{Bp} / (\text{Bp} + \text{H} + \text{R})$. The degree of field resistance for each of the eight progenies, as estimated by this parameter over the entire observation period was: P19A \times IMC67 (16.26%), ICS89 \times IMC67 (13.59%), PA13 \times IMC67 (13.33%), P19A \times PA150 (10.63%), PA13 \times PA150 (10.01%), PA121 \times IMC67 (9.81%), ICS89 \times PA150 (8.57%) and PA121 \times PA150 (8.16%).

Statistical analyses

The general linear model (GLM) in the SAS software (1989) was used for the analyses of variance. The mean score of leaf discs of one row in one tray was taken as the experimental unit. The data were corrected for any effect of the batches, where significant effects occurred, in order to obtain a better estimation of the genetic value of plants in the same progeny.

The genetic parameters of resistance (combining abilities, genetic variance, correlations between traits and heritability) were studied with DIOGENE software, an improved version of OPEP (Baradat & Labbé, 1995). The ten crosses of the 5×5 diallel and the 8 crosses of the 4×2 factorial were studied.

The diallel mating design was analysed using the Keuls and Garretsen general model (1977), with random effects (Cilas, 1995):

$$\text{Score}_{ijk} = \mu + g_i + g_j + s_{ij} + e_{ijk},$$

where, Score_{ijk} : score of plant k of cross female $i \times$ male j , μ : overall trial mean, g_i : general combining ability of parent i , g_j : general combining ability of parent j , s_{ij} : specific combining ability of cross $i \times j$, e_{ijk} : variance error s^2 .

The factorial design was analysed by the “North Carolina 2” design model, with random effects:

$$\text{Score}_{ijk} = \mu + f_i + m_j + c_{ij} + e_{ijk},$$

where, Score_{ijk} : score of tree k of cross female $i \times$ male j , μ : overall trial mean, f_i : random effect of female parent i with variance σ_f^2 , m_j : random effect of male parent j with variance σ_m^2 , c_{ij} : random effect of female $i \times$ male j interaction with variance σ_{fm}^2 , e_{ijk} : variance error σ^2 .

For field resistance, assessed through the percentage of rotten pods, only trees with a minimum total yield of 30 pods accumulated over the seven harvesting years were used for statistical analyses. This led to a variation of 25 to 30 trees actually analyzed per progeny. Genetic gains expected at different selection intensities were evaluated for the leaf disc test.

Results

Effect of reciprocal crosses

Six progenies of the 5×5 diallel design were studied involving two resistant parents, P7 and PA150, a moderately resistant parent, T60/887, and a susceptible parent, IFC1. Significant differences were found between the six types of progenies ($p < 0.001$; Table 1), but no significant difference was observed between the reciprocal crosses of each progeny (F value probabilities between 0.42 and 0.98). This justifies the study of the diallel mating design in one cross direction only (triangular diallel).

Effect of plant batches within inoculation series

The effects of plant batches were all non-significant for the four inoculation series of the diallel, but were significant for the factorial design. Correction for the batch

Table 1. Resistance scores in leaf disc tests of reciprocal crosses using parental clones with different level of susceptibility to *P. palmivora**

Reciprocal crosses studied			
P7 × PA150	1.99 a	P7 × IFC1	2.05 a
PA150 × P7	2.02 a	IFC1 × P7	2.08 a
P7 × T60/887	2.12 b	IFC1 × T60/887	2.09 a
T60/887 × P7	2.21 bc	T60/887 × IFC1	2.13 ab
PA150 × T60/887	2.25 c	IFC1 × PA150	2.18 bc
T60/887 × PA150	2.26 c	PA150 × IFC1	2.23 c

*R = Resistant (P7 and PA150), MR = Moderately Resistant (T60/887); MS = Moderately Susceptible (IFC1).

**Groups of significance according to Newman and Keuls test, at 5% probability.

effects was carried out for the progenies of the factorial mating design before genetic analyses were conducted.

Analysis of the mating designs

Statistical analysis of the diallel mating design revealed highly significant differences ($p < 0.001$; Table 2)

between clones and between progenies on the one hand, and between leaf test replicates (series of inoculations) on the other hand. Although significant, the “plant within clone” effect was very low ($p = 0.04$) compared to the “plant within progeny” effect ($p < 0.001$). The non-significant interaction effects between clones and test replicates on the one hand ($p = 0.44$) and between progeny and test replicate on the other hand ($p = 0.08$) showed that the relative resistance of the clones or progenies did not vary significantly from one replicate to the next. The same applied for individual plants within clones ($p = 0.62$) or within progenies ($p = 0.08$). This suggests that for young cacao trees placed under nursery conditions, it is possible to effectively estimate the degree of resistance of individual plants with a relatively small number of replicates.

Also with the factorial mating design, the effects of clones, of progenies and of test replicates were significant for the leaf susceptibility to *P. palmivora* ($p < 0.001$). The “tree within clone” effect was this time not significant ($p = 0.26$), but the “tree within progeny” effect was highly significant ($p < 0.001$). The interaction between “progenies” and “replicates” was not significant ($p = 0.16$). However, the interaction between

Table 2. Analyses of leaf resistance to *P. palmivora* of parental clones and of progenies of a 5×5 diallel and of a 4×2 factorial mating design

Source of variation	5 × 5 diallel				4 × 2 factorial			
	DF	MS	F	p	DF	MS	F	p
Parents								
Replicate	7	0.77	7.81	<0.001	8	2.66	18.13	<0.001
Parental clone	4	2.22	22.31	<0.001	5	4.85	32.93	<0.001
Plant within clone	16	0.17	1.77	0.04	10	0.18	1.27	0.26
Clone × replicate	28	0.10	1.03	0.44	40	0.28	1.89	0.01
Plant × replicate	28	0.09	0.89	0.62	16	0.08	0.52	0.92
Error	112	0.1			80	0.15		
Progenies								
Replicate	3	4.20	34.42	<0.001	3	7.10	44.81	<0.001
Progeny	14	3.38	27.72	<0.001	7	3.84	24.27	<0.001
Plant within progeny	406	0.44	3.48	<0.001	203	0.36	2.27	<0.001
Progeny × replicate	42	0.16	1.32	0.08	21	0.21	1.31	0.16
Plant × replicate	87	0.15	1.23	0.08	87	0.40	2.54	<0.001
Error	1218	0.12			606	0.16		

DF: degrees of freedom, MS: mean square, F: Fischers test and p: probability.

“clones” and “replicates” was significant ($p = 0.01$), though to a less significant degree than the clone effect itself ($p < 0.001$). The interaction effect between “trees within clones” and “test replicate” was not significant ($p = 0.92$), whereas it was highly significant ($p < 0.001$) between “trees within progenies” and “test replicate”. This showed that, leaves from clonal trees in the field (grown from cuttings and planted in rows) were less affected by environmental variations than leaves from hybrid trees planted in a totally randomized single-tree design.

Comparison between parental means and progeny means for leaf resistance

The results (Table 3) show that for seven out of the 10 hybrid progenies in the diallel, the mean of the parents was statistically identical to that of the progeny. With the factorial mating design, the mean of the parents was always statistically equal to that of their progeny, indicating already that additivity is the main genetic effect for the factorial mating design. On the other hand, for certain crosses of the diallel, effects of genetic dominance may have existed. These hypotheses were con-

Table 3. Comparisons between the clone parental means and cross-progeny means for resistance to artificial infections of leaf discs with *P. palmivora*

Mating design	Crosses	Parents	Progeny	p
5 × 5 Diallel	P7 × PA150	1.78	1.52	0.03
	P7 × T60/887	1.98	1.67	0.03
	P7 × H	1.84	1.83	0.94
	P7 × IFC1	2.02	1.84	0.10
	PA150 × T60/887	1.99	1.79	0.14
	PA150 × H	1.85	2.03	0.08
	PA150 × IFC1	2.03	2.08	0.62
	T60/887 × H	2.05	1.97	0.54
	T60/887 × IFC1	2.23	1.82	0.02
	H × IFC1	2.09	2.00	0.33
4 × 2 Factorial	PA121 × PA150	1.83	1.88	0.89
	PA121 × IMC67	2.26	1.97	0.29
	P19A × PA150	1.97	2.14	0.48
	P19A × IMC67	2.41	2.36	0.83
	PA13 × PA150	1.69	1.84	0.57
	PA13 × IMC67	2.12	2.08	0.86
	ICS89 × PA150	2.25	2.12	0.61
	ICS89 × IMC67	2.69	2.35	0.22

p : probability of the means of the parents and that of the progenies being equal.

firmed with the genetic analysis of these two mating designs (see below).

Genetic parameters of resistance assessed on leaf discs and effect of the number of replicates

The combining abilities and heritabilities of resistance were first estimated individually for each replicate of the leaf test conducted on different dates, then also for all possible combinations of the four replicates for both the diallel (Table 4) and the factorial design (Table 5).

The values of the ratio between CGA and SCA variances revealed a predominance of additivity effects over dominance effects, especially so for the factorial design. This result was clear for the four replicates conducted in the two mating designs, and did not increase significantly with the number of replicates involved. This therefore confirmed the previous results in the study on comparisons between the parents and their progenies (see above).

Table 4. Ratios between GCA and SCA variances ($\sigma_{GCA}^2/\sigma_{SCA}^2$), significance of CGA and SCA values and individual seedling heritabilities for resistance of leaf discs to *P. palmivora*, as a result of the number of replicates taken into account (5 × 5 diallel mating design without the selfed progenies)

Replicates	$\sigma_{GCA}^2/\sigma_{SCA}^2$	F_{GCA}	F_{SCA}	Heritabilities	
				Narrow sense	Broad sense
1	3.90	11.71***	3.00*	0.31	0.52
2	2.48	8.06***	3.24**	0.18	0.43
3	2.78	5.40**	1.94 ns	0.14	0.25
4	7.27	9.74***	1.34 ns	0.31	0.35
1 + 2	2.90	13.57***	4.67***	0.30	0.67
1 + 3	3.39	11.20***	3.30**	0.28	0.52
1 + 4	5.61	15.05***	2.68*	0.41	0.58
2 + 3	2.40	8.18***	3.41**	0.18	0.45
2 + 4	4.14	11.31***	2.73*	0.30	0.49
3 + 4	5.10	9.01***	1.77 ns	0.27	0.36
1 + 2 + 3	2.84	12.40***	4.37***	0.28	0.62
1 + 2 + 4	4.08	15.35***	3.76**	0.38	0.65
1 + 3 + 4	4.68	12.98***	2.77*	0.35	0.54
2 + 3 + 4	3.69	10.41***	2.82*	0.27	0.47
1 + 2 + 3 + 4	3.77	13.61***	3.61**	0.34	0.60

GCA = general combining ability; SCA = specific combining ability; F = Fishers test.

ns: not significant; *, ** and*** = significant at 5, 1 and 0.1%, respectively.

Table 5. Ratios of GCA and SCA variances ($\sigma_{GCA}^2/\sigma_{SCA}^2$), significance of CGA and SCA values, and individual seedling heritabilities for resistance of leaf discs to *P. palmivora*, as a result of the number of replicates taken into account (4×2 factorial mating design)

Replicates	Female parent		Male parent			Heritabilities	
	$\sigma_{GCA}^2/\sigma_{SCA}^2$	F_{GCA}	$\sigma_{GCA}^2/\sigma_{SCA}^2$	F_{GCA}	F_{SCA}	Narrow sense	Broad sense
1	7.96	4.30**	2.52	1.36 ns	0.54 ns	0.17	0.17
2	3.95	6.60***	12.22	20.42***	1.67 ns	0.34	0.39
3	11.40	14.01***	12.05	14.82***	1.23 ns	0.57	0.58
4	14.81	8.00***	16.59	8.96***	0.54 ns	0.36	0.36
1 + 2	6.20	8.50***	8.42	11.54***	1.37 ns	0.37	0.40
1 + 3	11.36	13.63***	8.42	10.11***	1.20 ns	0.55	0.56
1 + 4	54.78	9.86***	40.11	7.22**	0.18 ns	0.42	0.42
2 + 3	12.14	16.75***	21.56	29.75***	1.38 ns	0.67	0.70
2 + 4	13.19	10.95***	25.20	20.92***	0.83 ns	0.50	0.50
3 + 4	72.68	13.81***	85.21	16.19***	0.19 ns	0.58	0.58
1 + 2 + 3	10.85	16.17***	13.66	20.35***	1.49 ns	0.64	0.67
1 + 2 + 4	16.73	12.21***	21.66	15.81***	0.73 ns	0.52	0.52
1 + 3 + 4	45.88	15.14***	41.36	13.65***	0.33 ns	0.61	0.61
2 + 3 + 4	31.76	16.20***	39.31	20.05***	0.51 ns	0.67	0.67
1 + 2 + 3 + 4	24.84	16.89***	31.32	21.30***	0.68 ns	0.67	0.67

GCA = general combining ability; SCA = specific combining ability; F = Fishers test.
ns = not significant; *, ** and *** = significant at 5, 1 and 0.1%, respectively.

Narrow sense heritabilities, assessed on ten hybrids in the diallel mating design (i.e. on a total of 300 nursery seedlings) and on eight hybrids in the factorial design (i.e. on a total of 240 mature hybrid trees in the field) varied from 0.14 to 0.31 and from 0.17 to 0.57 respectively for the individual replicates. The corresponding broad sense heritabilities varied from 0.25 to 0.52 in the nursery and from 0.17 to 0.58 in the field, for the 4 replicates carried out, which revealed the substantial effects of the replicate in the part of the genetic variance explained in these mating designs. Combinations of 1 (4 possibilities), 2 (6 possibilities), 3 (4 possibilities) and 4 replicates of the leaf test led to average heritability values that increased in line with the number of replicates (Table 6). However, there was little increase in the heritability values with more than two replicates of the leaf test for the hybrid progenies in the nursery, and with more than three replicates for the hybrid progenies in the field.

For the averages of 4 test replicates, the narrow sense heritability value reached a limit of 0.34 and the broad sense heritability value reached 0.60 for the diallel (Table 6), indicating a certain dominance effect in that mating design. These two values were identical (0.67) for the factorial design, revealing a total absence of dominance effect.

Table 6. Mean heritabilities of leaf resistance to *P. palmivora* calculated from 1 to 4 test replicates in the 5×5 diallel (nursery) and 4×2 factorial (field) mating designs

Mating design	Heritabilities	Number of replicates			
		1	2	3	4
5×5 Diallel	Narrow sense	0.23	0.29	0.32	0.34
	Broad sense	0.39	0.51	0.57	0.60
4×2 Factorial	Narrow sense	0.36	0.51	0.61	0.67
	Broad sense	0.37	0.53	0.62	0.67

Combining abilities and heritability of field resistance

Field resistance was assessed from the rotten pod percentages on the hybrid trees in the 4×2 factorial design. For a better estimation of that parameter, only trees that produced at least 30 pods over the 1990 to 1997 period were taken into account, meaning a total of 232 trees were analyzed. The highly significant GCA effects for both type of parents and the non-significant SCA effects showed the superiority of GCA effects over SCA effects. The ratio between GCA and SCA variances was around 4 and 18, respectively, for the female and male parents (Table 7). Field resistance

Table 7. Ratios between GCA and SCA variances ($\sigma_{GCA}^2/\sigma_{SCA}^2$) and individual tree heritabilities, based on the percentage of rotten pods observed in the field over an 8-year period in the 4×2 factorial mating design

Female parent		Male parent		Heritabilities		
$\sigma_{GCA}^2/\sigma_{SCA}^2$	F _{GCA}	$\sigma_{GCA}^2/\sigma_{SCA}^2$	F _{GCA}	F _{SCA}	Narrow sense	Broad sense
4.05	7.33***	18.23	33***	1.81 ns	0.42	0.47

ns = not significant; *** = significant at 0.1%.

($\sigma_{GCA}^2/\sigma_{SCA}^2$ = ratio between GCA (general combining ability) and SCA (specific combining ability)).

heritability was 0.42 in the narrow sense and 0.47 in the broad sense.

Genetic gains expected with the leaf disc test

Genetic gains were calculated for each mating design for selection intensities of 5, 10, 20 and 50%, based on the results obtained with 4 replicates on leaf discs. Genetic gain, expressed as a percentage of the mean of the base population obtained at the 5% rate (Table 8), was -29.70% in the diallel and -29.11% in the factorial design. As the average susceptibility score for the base population was 1.79 in the diallel and 1.82 in the factorial design, the relative genetic gains adopted for the 5% rate in each mating design were used to calculate an expected genetic gain (relative gain \times base population mean), i.e. a 0.53 reduction of the average susceptibility score of the base population. The expected means of the selected populations would therefore be 1.26 (1.79–0.53) in the diallel mating design and 1.29 (1.82–0.53) in the factorial mating design. This is lower than the mean scores of the most resistant parental clones in the diallel (P7 = 1.67) and in the factorial (PA150 = 1.40).

In the diallel, the most resistant plants identified in the leaf disc test belonged to progenies P7 \times PA150 (6 plants), P7 \times T60/887 (4 plants), P7 \times H (1 plant) and T60/887 \times IFC1 (1 plant). For the most resistant trees in the factorial design, four were from the progeny PA13 \times PA150 and three from the progeny PA121 \times PA150. This revealed the ability of P7 and PA150 to transmit high resistance levels to their progenies.

Genetic gains expected based on natural infection in the field

Using the average level of natural infection observed in the factorial design, the expected genetic gain at 5% selection intensity was calculated to be 18%. With an average level of field infection of 11.3% observed in the trial, over a seven-year period, this would correspond to a decrease in field infection for the selected population with 2%, to 9.3%. This would seem a relatively modest improvement, as this is still more than the average infection level of the best hybrid tested in the factorial trial (8.2%).

Table 8. Expected genetic gains for leaf resistance to *P. palmivora* assessed in the 5×5 diallel and the 4×2 factorial mating designs

Mating design	Selection rate (%)	R.G.G (%)	E.G.G (%)	Expected mean score (selected population)	Mean leaf susceptibility score (of base population)
5 \times 5 Diallel	5	-29.7	-53.1	1.26	1.82
	10	-25.3	-45.2	1.34	
	20	-20.2	-36.1	1.43	
	50	-11.5	-20.6	1.59	
4 \times 2 Factorial	5	-29.1	-53	1.29	1.79
	10	-24.8	-45	1.37	
	20	-19.7	-36	1.46	
	50	-11.3	-20	1.62	

R.G.G. = Relative Genetic Gain, E.G.G. = Expected Genetic Gain.

Table 9. Coefficients of correlation (r) between the percentages of rotten pods on trees in the field and the susceptibility scores evaluated on leaf discs from trees in the 4×2 mating design

Genetic	Phenotypic	Environmental
Coefficients of correlation		
0.74 *	0.39 ***	0.02 ns

Significance thresholds:

Genetic correlation at 5% (*) = 0.70.

Phenotypic or environmental correlation at 5% (*) = 0.19 and at 0.1% (***) = 0.32.

ns = not significant.

Relations between responses to artificial infections on leaves and responses to natural infections on pods

The coefficient of genetic correlation between leaf susceptibility scores and the percentages of rotten pods in the field was positive and significant, with a value of 0.74 (Table 9). The phenotypic coefficient of correlation (simple correlation for all the trees studied) was also positive and highly significant, although the value obtained (0.39) was lower. The environmental coefficient of correlation was 0.02.

Discussion

Effect of the number of replicates on progeny or clone selection efficiency

In each of the two mating designs, the clones and progenies displayed significant differences in resistance when 4 leaf disc test replicates were used. This suggests the possibility of efficiently selecting clones and progenies using the leaf disc test and confirms results obtained earlier, by Nyassé et al. (1995) and by Tahí et al. (2000).

The absence of any interaction between replicates and progenies (Tables 2 and 3) showed that the average level of resistance in the progenies or clones could already be estimated with a degree of accuracy from a single replicate. This was probably due to the large number of plants (30) tested within the progenies, be it in the nursery or in the field. For routine selection work on progenies, at least two replicates could be recommended, with the number of plants varying between 15 to 30 per progeny.

For clones, the interaction between replicates and clones was significant for the factorial design, where the number of trees per clone was limited to three. For

the diallel, the interaction was not significant, with 5 plants per clone in the nursery. In order to estimate accurately the average resistance level of a clone, it thus seems best to use at least 5 plants per clone, with two or three replicates.

Effect of the number of replicates on plant selection efficiency within progenies

A significant effect was found for plants within progenies for the diallel (nursery plants) and factorial design (trees in the field), using 4 replicates. A significant effect of plants within hybrid progenies had already been found in earlier work (Tahí et al., 2000). This suggests that it is possible to select individuals within progenies right from the nursery stage for their lower susceptibility to *P. palmivora*.

It is worth pointing out that the heritabilities obtained for individual replicates were highly variable (Tables 4 and 5). For broad-sense heritability, these values varied from 0.25 to 0.52 for the diallel, and from 0.17 to 0.58 for the factorial design. This indicates that some replicates were conducted under better conditions than others for expression of genetic variation.

Average heritability increased with the number of replicates (Table 6). This shows that the share of genetic variance increases with the number of replicates. High heritability levels were already obtained with 2–3 replicates. For efficient selection of individual plants within progenies, it thus seemed preferable to use a minimum of 3 replicates.

Genetic analyses

The lack of a reciprocal effect on resistance assessed on leaves suggests that this trait does not show cytoplasmic inheritance. This result is in agreement with those obtained by Rodríguez et al. (1985) in Costa Rica and by Despréaux et al. (1989) in Cameroon, who both found in studies of complete diallel designs that transmission of resistance to black pod in the field did not display any reciprocal effect either.

A genetic analysis of resistance assessed on leaf discs in the two mating designs revealed that general combining ability (GCA) effects predominated over specific combining ability (SCA) effects. These results are in agreement with other publications (Cilas et al., 1998; Iwaro et al., 1997; Nyassé et al., 2002; & Flament, 1998). Moreover, the additivity of leaf resistance was confirmed by identical broad-sense and

narrow-sense heritability values for the factorial mating design (0.67).

However, for the diallel the SCA effect was also significant, although the GCA effect predominated. This is reflected otherwise through quite substantial differences between the narrow-sense heritability value (0.34) and the broad-sense heritability value (0.60). Significance of non-additive effects was also reported by Iwaro et al. (1997) for resistance to penetration (lesion frequency) of *P. palmivora* in cacao tree leaf tissue.

The heritability of resistance in the field (based on natural infection) assessed on 232 trees was 0.42 in the narrow sense, and 0.47 in the broad sense. As for leaf resistance measured in the factorial design, the GCA effect was not significant. This tallies with the conclusions of various authors on resistance to *P. palmivora* measured by natural infection of fruits (Tan & Tan, 1990; Berry & Cilas, 1994; Nyassé, 1997).

The higher heritability values assessed on artificially infected leaves (0.67) compared to the heritability values measured on naturally infected pods (0.42 and 0.47) could be explained not only by the type of organ concerned (leaves in the laboratory and fruits in the field), but also by the impact of environmental variations on trait expression, which is supposedly greater in the field than in the laboratory. Selection based on the artificial inoculation test could therefore be more efficient than selection based on seven years of field observations. The efficiency depends further on the correlation between the laboratory test and field observations (see below).

Correlation between field results and laboratory results

The comparison of the eight progenies in the factorial design for their level of resistance in the field and in the laboratory revealed a significant genetic correlation (0.74). This result tallies with the results already published by Tahi et al. (2000). The phenotypic correlation based on individual tree data was also significant but lower, as expected. These results confirm that selection based on leaf resistance can be effective in reducing black pod disease levels in the field.

Estimated genetic gains

The genetic gain in leaf resistance estimated for a 5% selection intensity was approximately 30% for both mating designs. This compares to gain of 18%,

observed for the same selection intensity, when seven years of data on natural infection in the field would be used as selection criterion. The difference can be explained by the relatively lower heritability for the field resistance in relation to the heritabilities observed for the leaf disc tests, as demonstrated in this article. In view of the significant linear coefficients of correlation between the scores in the leaf disc test and the level of natural level of infection in the field, up to values of 0.7 to 0.9 (see also Tahi et al., 2000; Tahi, 2003), it seems reasonable to expect that early selection based on leaf resistance can indeed be very effective to reduce pod rot infection levels in the field.

Conclusion

The results show that it is possible to apply effectively early screening for *Phytophthora* resistance of cocoa, gaining substantially in time and probably also in efficiency, as compared to selection based on natural infection only.

The primarily additive transmission of leaf resistance to *P. palmivora* suggests that the apparent resistance of clones can be a good predictor of their combining ability. This makes it possible to use the clone value to predict the average resistance of seedling progenies, obtained by crossing parental clones with known levels of resistance.

The test based on leaf disc inoculations can be conducted to select efficiently the best progenies using two replicates (series of inoculations), by applying the methodology used in this work. When using the test for early screening, the best nursery progenies can be planted out in the field so that other worthwhile traits can be studied.

If the aim is clonal selection, it will be possible to clone immediately the best plants identified within field or nursery progenies, based on 3 or 4 series of inoculations. Buddings, graftings or rooted cuttings may be used for the clonal multiplication of selected field trees or nursery seedlings. Such clones could be further evaluated in field observation or selection trials, together with clones that have already shown good potential for resistance to *Phytophthora* pod rot.

In addition, the availability of reliable resistance assessment tests is fundamental to gain better understanding of the genetic determinism of resistance to *Phytophthora* pod rot of cocoa, notably the number of genes involved and their location on the genome (Lanaud et al., 1999).

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