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Integrated management of *Phytophthora* diseases on cocoa (*Theobroma cacao* L): Impact of plant breeding on pod rot incidence

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Abstract

Pod rot, caused by several species belonging to the genus *Phytophthora*, is the main cause of cocoa harvest losses worldwide. Among the methods making up integrated disease management (IDM), the creation of resistant cultivars has been identified as a priority in cocoa breeding research programmes. To that end, various experiments have enhanced knowledge about the genetic basis of resistance to pod rot. Genetic trials conducted in Cameroon, Ivory Coast and Togo indicated that genetic × environment interactions were relatively low. Rankings of progenitors tested were stable in different conditions, from one country to another. The greater the number of years of field observations, the higher the heritability of the pod rot resistance trait. A protocol for early evaluation of disease resistance on leaf discs has been developed and validated for the selection of more resistant families. The leaf disc test developed was well correlated at the genetic level to the pod test previously used. Heritability of mean disease scores obtained with the leaf disc test after several inoculation rounds is similar to the one of pod rot rate in the field after several years of observations. The potential use of the leaf disc test as breeding tool and its impact on the genetic improvement of black pod resistance are discussed.

Keywords: Cocoa; *Phytophthora* sp.; Disease resistance; Integrated disease management; Breeding tool

1. Introduction

The deforestation seen in most cocoa-producing countries has reached limits that are prejudicial to ecological balances, and cropping systems on cleared forest land are no longer acceptable. Securing cocoa supplies will therefore have to involve sustainable management of the ecosystems in which cocoa trees are included. Such sustainable management will require rational control of the main health threats: diseases and pests.

Among the diseases affecting cocoa plantations, pod rot caused by various species belonging to the genus *Phytophthora*, is the main cause of harvest losses worldwide (Despréaux et al., 1989). Chemical control of this disease is laborious and usually not cost-effective; moreover, it does not fit in with a sustainable process of cocoa plantation management. Chemical treatments, which have a limited effect, usually entail numerous constraints for farmers, notably the cost of fungicides and treatment equipment, water supply, and excessive treatment frequency, notwithstanding pollution. Of the alternatives to chemical control, genetic improvement of planting material appears to be a promising avenue. Selecting cocoa trees displaying lower susceptibility to pod rot has therefore become a priority objective for numerous producing countries.

Despite a great deal of work (Thorold, 1953; Tarjot, 1969; Blaha and Lotodé, 1976), the search for cocoa trees displaying total resistance to this disease has drawn a blank. Numerous authors have reported that differences in reaction to *Phytophthora* spp. are down to partial, probably polygenic resistance (Partiot, 1975; Blaha and Lotodé, 1977). Different ways of assessing planting material have been tested (Blaha, 1974). Observations of field performance under natural infection conditions and artificial inoculation tests on fruits or leaves remain the

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main methods adopted (Nyassé et al., 1995; Iwaro et al., 1998). The validity of artificial inoculation tests relies on their correlation with the pod rot rates observed in the field (Nyassé, 1997). Studies based on field observation of pod rots have revealed the existence of genetically transmittable partial resistance (Despréaux et al., 1989). Transmission of that trait is mainly additive (Cilas et al., 1995).

An international project was launched to examine the possibilities of breeding cocoa trees for resistance to this disease. The aims of this project were to:

- identify the factors involved in resistance,
- develop and validate resistance tests,
- localize the zones of the genome involved in the resistance trait by seeking quantitative trait loci (QTLs),
- start a process of breeding for that trait.

The most significant results of all this work are presented and the integration of cultivar improvement in integrated disease management is discussed.

2. Material and methods

2.1. Resistance measured by the rotten pod rate in the field

The data used came from several mating designs set up in zones affected by the disease in producing countries: Cameroon, primarily with *P. megakarya*, Togo with *P. megakarya* and *P. palmivora*, and Ivory Coast, where *P. palmivora* is predominant, but which is threatened by the spread of *P. megakarya* in the east of the country (Nyassé et al., 1999).

In Cameroon, the cocoa trees observed all came from a 6×6 complete diallel mating design (without the selfs). This trial, which was planted at the Barombi-Kang station in 1974, comprised six blocks. In each block, 12 trees per family were planted in a totally randomized manner with a density of 1330 plants/ha. The six parents used were: SNK 10, UPA 134, IMC 67, ICS 95, SNK 413 and ICS 84; SNK 10 and SNK 413 were local Trinitario trees, ICS 95 and ICS 84 were Trinitario trees from Trinidad bred by ICTA, UPA 134 was a clone derived from an Upper Amazon (UA) Forastero progeny from Ghana and IMC 67 was an UA collected at Iquitos (Peru).

In Togo, the trees under observation came from a 12×12 triangular diallel mating design (without the selfs). The plot, which was planted in 1987 in the Litimé region, comprised two blocks. In each block, six trees per family were planted in a totally randomized manner, with a density of 1330 plants/ha. The 12 parents used were composed of three UA clones of wild origin (Sca 6, IMC 67, Na 32), three UA clones derived, respectively, from crosses Pa 7 × Na 32 (T60/887); IMC 60 × Na 34 (T85/799) and Pa 35 × Pa 7 (T86/45), one UA progeny bred in Ghana (UPA 34), two Lower Amazon (LA) clones bred, respectively, in Ivory Coast (IFC 5) and in Cameroon (SNK 64), and three Trinitario clones bred in Costa Rica (UF676) and in Trinidad (ICS 40 and ICS 100).

In Ivory Coast, the cocoa trees under observation came from a factorial mating design of crosses between 16 UA female parents and four LA male parents. The trees of the different crosses were planted in a totally randomized design of single-tree plots. The 16 female parents used were composed of eight UA of wild origin (P 7, Na 79, Sca 6, IMC 67, Pa 150, Na 32, Pa 7, IMC 78), UA derived from crosses Pa 7 × Na 32 (T60/887), Na 32 × Pa 7 (T79/501, T79/416 and T79/467), Pa 35 × Na 32 (T63/971 and T63/967), IMC 60 × Na 34 (T85/799) and one poorly identified Trinitario (Pa 35*). The four male parents used were LA bred locally (IFC 1, IFC 2, IFC 5, IFC 15).

Each tree in the trial was observed over the cropping period (May–November) for 7 years in Cameroon (1986–1990, 1995 and 1996), in 1991 in Togo and for 9 years in Ivory Coast (1983–1992).

Pods that were rotten (affected by black pod) wilted (early desiccation of a physiological nature) damaged by rodents, and ripe and healthy, were counted weekly. During the counts, sanitation harvesting was carried out: all pods except unripe healthy pods were removed. The topographical position of the different trees was also recorded in Togo, making it possible to display pod rot distribution in the plot (Cilas et al., 1999a)

Losses caused by pod rot were estimated in relation to potential production (excluding rodent-damaged pods) using the formula giving the pod rot rate per tree (Rrot):

$$\text{Rrot} = \frac{\sum \text{rotten pods}}{\sum \text{rotten pods} + \sum \text{ripe pods} + \sum \text{healthy pods on last count}}.$$

Statistical analyses of the mating designs were performed with Diogène software (Baradat and Labbé, 1995). Narrow-sense and broad-sense heritability values were estimated for the three countries. These estimations were based on pod rot rates per tree.

2.2. Leaf tests and pod tests

The leaf test is a method of artificial leaf inoculation used to assess genotype resistance. The test was first developed on whole leaves and was then applied to leaf discs 15 mm in diameter, in order to reduce the space needed to compare genotypes. Leaf discs were placed in trays and then inoculated with 10 μl drops of a *Phytophthora* sp. zoospore suspension. Observations were carried out 5 and 7 days after incubation at 26 °C, scoring on a scale of 0–5 (Nyassé et al., 1995).

The pod test measures the diameter of rot lesions on fruits artificially inoculated with a calibrated zoospore suspension (Blaha, 1974; Iwaro et al., 1998). Inoculations may be carried out on fruits still on the tree or on loose fruits, with or without wounds to the epidermis. The same planting material was tested by this method to estimate correlations between these different assessment methods.

Correlations between the leaf test and the pod test were estimated from a trial comparing 12 clones, comprising several plots located at the IRAD research station (Kumba/Barombi-Kang, Cameroon). They were clones SNK10, SNK13, SNK30, SNK64, SNK413, ICS1, ICS84, ICS95, UPA134, UPA143, T79/467 and Sca12. Multivariate analyses of variance were carried out to determine several types of correlations: phenotypic, genotypic and environmental (Baradat et al., 1995).

The heritability of the scores obtained with the leaf test was estimated from different trials in Ivory Coast. The first trial was a triangular diallel involving five parents with the selfs planted in the nursery at the CNRA station in Bingerville. The parents used were: P7, PA150, T60/887, IFC1 and a tree of the UPA402 × UF676 hybrid identified for its resistance to *P. palmivora*, assessed on leaves. The assessment involved 30 plants of each progeny in two series of inoculations over time. Each series consisted of two batches of 250 plants each, and for each batch 15 plants were tested per family. The second trial was a 4 × 2 factorial mating design. Eight progenies, all consisting of adult trees, were planted in an experimental plot at the CNRA station in Bingerville in a randomized single-tree plot design. The female parents were UA and Trinitarios (T), while the male parents were two UA. The same assessment protocol was used as in the diallel trial.

2.3. Comparison between resistance tests and rot rates recorded in the field

Correlations between the resistance tests (on leaves and pods) and rot rates in the field were estimated on five plants in a plot located on the IRAD station at Kumba/Barombi-Kang. This involved clones SNK10, SNK413, ICS84, ICS95 and UPA134. Pearson and Spearman's coefficients were estimated.

3. Results

3.1. Resistance measured by the rotten pod rate in the field

The analyses of variance carried out on the mating designs showed that general combining abilities (GCA) dominated, meaning that transmission of the resistance trait was primarily additive. The parents were classed according to the rot rate observed in their progenies. It involved a multiple comparison of GCAs estimated by parent (Table 1). All in all, the parent classifications tallied between the three countries despite the different pathogen species. Thus, selection carried out in Ivory Coast for resistance to *P. palmivora* will be useful in the event of *P. megakarya* spreading to that country. Trinitario parents were generally more susceptible to the disease. Amelonado-type LA and some UA parents, such as Sca 6, P 7, Pa 150 or T85/799 should help in creating less susceptible cultivars.

Table 1
Classification of the different parents for their susceptibility to black pod rot (Newman and Keuls test (5%))

	Cameroon *P. megakarya* (data over 3 years)	Togo *P. megakarya* (data over 1 year)	Ivory Coast *P. palmivora* (data over 9 years)
−susceptible ↑			
		IFC 5 a	Pa 150 a
		SNK 64 ab	Sca 6 a
		T86/45 ab	P 7 a
		T85/799 ab	T85/799 b
		T60/887 ab	T60/887 bc
		Sca 6 ab	T79/416 bc
		ICS 100 ab	T79/501 bc
	UPA 134 a	UPA 134 abc	Pa 7 bcd
	SNK 413 b	Na 32 abc	Na 32 bcde
	ICS 84 bc	UF 676 bc	
	IMC 67 bc	IMC 67 c	IMC 67 bcde
	ICS 95 bc		T63/971 bcde
	SNK 10 c		T79/467 bcde
			Na 79 cde
			IMC 78 de
+susceptible			

Table 2
Narrow-sense heritability (h^2) and broad-sense heritability (H^2) of rotten pod rate

	Pathogen species	Number of observation, years	h^2	H^2
Cameroon	*P. megakarya*	7	0.155	0.195
Togo	*P. megakarya*	1	0.061	0.061
Ivory Coast	*P. palmivora*	9	0.681	0.681

Broad-sense heritability values were identical to the narrow-sense heritability values for Togo and Ivory Coast, and about the same for Cameroon (Table 2). Heritability of primarily the additive type therefore seemed to govern the transmission of this trait, which confirmed earlier studies (Tan and Tan, 1990; Berry and Cilas, 1994). The heritability values increased in line with the number of years taken into account. The precision of the genetic values was in fact better when observations were carried out over a larger number of years. In that respect, data from the Ivory Coast experiment should be considered the most reliable, while further observations should be carried out in Togo.

Genetic and environmental correlations between rot rates and potential yields were calculated, assessed by the total number of pods produced per tree (Cilas et al., 1998). Genetic correlations between potential yields and rot rates were favourable (negative). It is therefore possible to carry out combined selection for those two traits. Combined "individual-family" selection based on an index combining high yields and good resistance to rot has been proposed for Cameroon and Ivory Coast, in order to select from worthwhile families those individuals suitable for use as clones or as parents for new crosses (Cilas et al., 1995,

Table 3
Phenotypic, genetic and environmental correlations between leaf and pod tests

	SL3			SL5			SL7			INSL		
	P	G	E	P	G	E	P	B	E	P	G	E
SP3	0.24	0.64	−0.25	0.26	0.57	−0.23	0.32	0.57	−0.15	0.34	0.50	−0.10
SP5	0.32	0.81	−0.29	0.37	0.79	−0.30	0.42	0.78	−0.22	0.48	0.77	−0.02
SP7	0.29	0.85	−0.32	0.37	0.86	−0.31	0.44	0.84	−0.23	0.51	0.85	−0.05
INSP	0.28	0.86	−0.31	0.37	0.89	−0.31	0.44	0.87	−0.24	0.52	0.91	−0.03

Correlation threshold (5%), P, phenotypic correlations; G, genetic correlations; E, environmental correlations.
SL3, symptom score on leaves 3 d after inoculation; SL5, symptom score on leaves 5 d after inoculation; SL7, symptom score on leaves 7 d after inoculation; INSL, increase in symptoms on leaves between 3 and 7 d; SP3, symptom score on pods 3 d after inoculation; SP5, symptom score on pods 5 d after inoculation; SP7, symptom score on pods 7 d after inoculation; INSP, increase in symptoms on pods between 3 and 7 d.

1999b; Ndoumbè-Nkeng et al., 2001). A field trial has been set up in Cameroon to confirm the selected material.

3.2. Leaf tests and pod tests

3.2.1. Correlations between leaf tests and pod tests

The data for leaf and pod tests carried out in a trial comparing clones were analysed using a multivariate analysis of variance, in order to determine the different correlations. ‘‘Clone’’ effects were significant for each of the traits studied. Phenotypic correlations between leaf tests and pod tests were positive and significant (Table 3). Based on a multivariate analysis of variance, it was possible to calculate genetic and environmental correlations between these different variables (Cilas et al., 1998). The genetic correlations corresponded to correlations between means per clone. They were systematically positive between leaf and pod tests (Table 3). However, these correlations were not always significant, as they were calculated on too small a number of clones (five clones). On the other hand, the environmental correlations between leaf and pod tests were negative (Table 3). There were therefore environmental effects on the expression of the tests, and those effects differed depending on the organ tested.

3.2.2. Heritability of the resistance trait estimated by the leaf test

In the two mating designs studied in Ivory Coast, the heritabilities (narrow sense and broad sense) of the scores obtained by tests on leaves inoculated with *P. palmivora* were calculated. Heritability values increased in line with the number of series taken into account as the precision of the genetic values was better when plants were assessed several times (Tables 4 and 5). However, the values obtained did not vary significantly between broad-and narrow-sense heritability. The resistance trait estimated in that way was therefore transmitted additively.

3.2.3. Correlations between resistance tests and rot rates in the field

In terms of the relations between leaf test results and rot rates observed in the field, no strong phenotypic correlations (tree per tree) were found, be it in the clonal trials or the hybrid trials (Table 6) (Nyassé, 1997). However, a positive genetic correlation was often detected between rot rates and pod tests, and especially leaf tests. Consequently, the mean values per clone or per hybrid were correlated and it was therefore possible to select a clone or cross based on leaf tests carried out in an appropriate experimental design (Nyassé et al., 2002). Selecting genotypes within a progeny is not yet reliable with leaf tests and further experiments are required before considering this method for the early selection of individuals (Cilas and Despréaux, 2004).

Table 4
Individual heritability values for resistance to *P. palmivora* measured on leaves in two series of inoculations (S1, Series 1; S2, Series 2)—diallel with five parents

Heritability	S1	S2	S1 + S2
Broad sense	0.490	0.427	0.662
Narrow sense	0.294	0.203	0.320

4. Discussion

This genetic study of cocoa tree resistance to *Phytophthora* pod rot diseases in plots in Cameroon, Togo and Ivory Coast confirmed the existence of mainly additive transmission for the trait under natural infection conditions (Despréaux et al., 1989; Tan and Tan, 1990). In fact, analyses of variance for these three designs indicated that GCAs were dominant, meaning that resistance trait transmission was primarily additive. Classification of the parents tallied among the three countries despite different pathogen species. Selection undertaken for resistance to one *Phytophthora* species could be used for the improvement of the resistance of others species. The Trinitario parents were generally the most susceptible to the disease. The length of the fruiting cycle in that material may contribute to its poor performance in the field (Berry and Cilas, 1994). The Amelonado-type Lower Amazon parents and some Upper Amazons such as Sca 6, P 7, Pa 150 or

Table 5
Individual heritability values for resistance to *P. palmivora* measured on cocoa tree leaves in the field; results of three series of inoculations (S1, Series 1; S2, Series 2; S3; Series 3)—4 × 2 factorial design

Heritability	S1	S2	S3	S1 + S2	S1 + S3	S2 + S3	S1 + S2 + S3
Broad sense	0.103	0.325	0.581	0.248	0.451	0.666	0.685
Narrow sense	0.103	0.292	0.565	0.248	0.451	0.657	0.662

Table 6
Analysis of correlations between resistance assessed by pod tests and resistance estimated by leaf tests, and the rot rates recorded in two plots

Factors	LT3	LT5	LT7
Plot A			
RP1 (30 trees)	−0.26	−0.23	−0.22
RP2 (31 trees)	−0.11	−0.16	−0.13
RR (32 trees)	0.24	−0.25	0.21
Plot B			
RP1 (28 trees)	−0.43*	−0.43*	−0.48*
RP2(28 trees)	−0.43*	−0.43*	−0.41*
RR (29 trees)	−0.02	−0.05	−0.12

RR, rot rate; RP and RP2, lesion growth rate on pods between the third and seventh day in the first series of inoculations (RP1) and during the second series of inoculations (RP2). LT3, LT5, LT7, result of the leaf test on the third, fifth and seventh day.

T85/799 should help in creating less susceptible cultivars, notably in Cameroon, where those parents were introduced recently (Nyassé et al., 2003).

Resistance tests on leaves and pods in an appropriate experimental design can be used to select less susceptible clones or hybrid families. However, improvements are needed if this assessment technique is to be used to select resistant individuals within hybrid families. In that case, the uniqueness of the genotypes tested requires robust experimental designs. Likewise, rot rates estimated individually on trees in trials cannot be used to precisely determine the degree of susceptibility of those trees. The observed rot rate may depend on the immediate environment of the tree, its pod load, the layout of the pods, etc. Indeed, field observations depend on many environmental factors that are difficult to control with conventional experimental designs.

In order to estimate the reliability of the leaf test, we suggest applying it in the nursery and validating it by observing the field performance of material tested in that way at an early stage. It is now a matter of validating these assessment techniques on a true scale, under real breeding conditions, and of estimating genetic gains in the rot rate for different selection rates determined from leaf disc tests. Early selection based on leaf disc tests could then be pursued. Detecting the zones of the genome involved in resistance to *Phytophthora* diseases is a further line of research that provides a clearer understanding of trait transmission (Lanaud et al., 1997, 1999, 2004; Flament, 1998), and this could be useful in detecting the same zones involved in resistance assessed by the leaf disc test and pod rot rate in the field.

This study has enhanced knowledge of the genetic basis of resistance to *Phytophthora* diseases. Material displaying greater resistance has been selected in the partner countries. It is now necessary to continue the trials already under way to confirm the selected material. Breeding schemes using these first results should be implemented in order to contribute to the current genetic improvement for the resistance trait. Lastly, planting less susceptible material in bearing plots is the first step towards integrated disease management. This material, grown with slight shading and appropriate sanitation harvests, should not require as many fungal treatments as the planting material currently grown.

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