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Luteolin derivatives and heritability of resistance to *Phytophthora megakarya* in *Theobroma cacao*

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Abstract. Diseases are a major production constraint wherever cocoa (*Theobroma cacao*) is grown. The principal method of ameliorating cocoa production is the use of hybrid clones, which have resistance to important diseases and high productivity. To select new genotypes with resistance to *Phytophtora megakarya*, the cause of the destructive black pod disease, comparative analyses (quantitative and qualitative) of phenolics were conducted on the leaves of parental genotypes considered tolerant (SNK413) or sensitive (SNK10) to black pod disease and hybrids (families F16 and F3) derived from reciprocal crossbreeding between these two parental clones. A negative correlation between the size of necrotic lesions and the total phenolic content was demonstrated. Three individuals of the F16 family (F1612, F1614 and F1627), progenies from the ♀ SNK413 × ♂ SNK10 cross, had small lesions and high concentrations of phenolics. The F1612, F1614 and F1627 genotypes, which had performances similar to those of the tolerant parent SNK413 can be considered to be elite clones. The heterosis linked to containment of lesions revealed the manifestation of strong hybrid vigour for the genotype F1612 followed by F1614 and F1627. However, after analysis of total phenolics, no maternal effect was detected in the transmission of this character. Qualitative analyses of phenolic compounds by high performance liquid chromatography from the parents and individuals from the two families showed the accumulation of luteolin derivatives after inoculation in the SNK413 clone and individuals where the female parent was the SNK413 clone. This may indicate that some resistance characters of cocoa to *Phytophthora* spp. are cytoplasmic. These compounds among many other unidentified compounds have an essential role in the reaction and mechanism of defence of cocoa against *P. megakarya*.

Introduction

Cocoa (*Theobroma cacao* L.) is an important crop in many tropical countries (Brazil, Ghana, Côte d'Ivoire, Nigeria and Cameroon). Global annual earnings from exports of cocoa average US$2.9 billion (Gray 2000). Cocoa production is confronted by many problems including low yields and the impact of plant diseases. Diseases include black pod, caused by several *Phytophthora* species, frosty pod, caused by *Crinipellis roreri*, and witches broom, caused by *Crinipellis perniciosa* (Wood and Lass 1985). In Cameroon, *Phytophthora megakarya* is the major cause of black pod (Nyassé *et al*. 1995; Omokolo *et al*. 2002; Omokolo *et al*. 2003). In areas where *P. megakarya* is present, 50% of the crop is rendered unusable. Losses may be as high as 80% when conditions favour disease development (Despréaux *et al*. 1989). Fungicides are used to control the disease and mummified pods are removed at the beginning of the season, followed by weekly phytosanitary removal. Chemical control is expensive, commercially non-viable and environmentally harmful. Resistance to pod rot has become a major breeding target.

In Cameroon, most of the cultivated cocoa trees were originally derived from old varieties introduced by German colonists, and from second generation seeds obtained from new hybrid cultivars. Hybrid selection was based on heterosis observed in crossing genetically distinct genotypes. Local and introduced clones available in clonal banks were generally used as hybrid progenitors. Due to their yield capacity and environmental adaptation, vigour of these hybrids has been used on a large scale. However, these hybrids have generally not been satisfactory with regards to disease resistance and yields have often declined after 5–10 years. Moreover, these hybrid varieties have also shown large phenotypic variation for all traits and are not accepted by farmers (Ndoumbe-Nkeng *et al*. 2001). Cocoa breeders continue to face the problem of high heterogeneity between individuals derived from one cross and heterogenous transmission of genetic traits to the progeny.

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Hybridisation involves heterosis combined with productivity and vigour. The F1 product of a crossbreeding between two varieties of the same species developed vigour higher than that of the parents. This 'hybrid vigor' manifested by increasing size, growth rate or other parameters resulting from the increase in heterozygosity in F1 generation crosses between inbred lines (Gallais 1990). Djocgoue (1998) and Nyasse (1997) showed that there is a correlation between the resistance of cocoa to *P. megakarya* and the size of necrotic lesion after artificial inoculation screening of pods and leaves for disease development. Little is known about the molecular and physiological responses of *T. cacao* to pathogen infection.

The role of phenolic compounds in plant defence is well documented (Tan *et al*. 2004; Omokolo and Boudjeko 2005). Generally, phenolics accumulate at different levels in infected tissues in response to pathogen invasion. The resistance of apple (*Malus domestica*) to *Ventura inaequalis* is related to the higher content of catechin and proanthocyanidins in leaves (Treuter and Feucht 1990). Daayf *et al*. (1997) reported the accumulation of a methylester, *p*-coumaric acid, in leaves of cucumber (*Cucumis sativus*) infected by *Sphaerotheca fuliginea*. In the date palm–*Fusarium oxysporum* f. sp. *albedinis* pathosystem, there is a higher accumulation of non-constitutive hydroxycinnamic acid derivatives in resistant cultivars (El Hadrami *et al*. 1997). Some plants can inducibly form a large variety of phenolic phytoalexins. Kodama *et al*. (1992) listed some 16 phytoalexins produced by rice (*Oryza sativa*) in response to pathogen attack.

This study investigates the resistance of cocoa progenies (F16 and F3) from SNK413 × SNK10 crosses for resistance to *P. megakarya* by measuring the size of necrotic leaf lesions following field inoculation. We determined the quantitative and qualitative composition of phenolic compounds in healthy and wound-inoculated leaves of these genotypes and analysed the heritability of resistance to *P. megakarya* using this data.

Material and methods

Cocoa plant material

Two cocoa clones (SNK413 and SNK10) from the gene banks at the Institute of Agricultural Research for Development (IRAD) at the Nkoemvone Research Station (Southern Cameroon) were used to create two hybrids. Clones were crossed at the Nkoemvone Research Station of IRAD in March and April 2000 using hand-pollination (Cilas 1991). Hybrids obtained were F16 (♀ SNK413 × ♂ SNK10) and F3 (♀ SNK10 × ♂ SNK413).

Production of seedlings and grafts

Seeds from parent clones harvested in an experimental field were sown in the nursery at Teacher's Higher Training College of the University of Yaoundé (Cameroon), to obtain hybrid plants. Parental plantlets were obtained through top-grafting by using bud wood from the two clones listed above. This grafting was done on non-specific young cocoa plantlets.

Leaf inoculation and analysis

A leaf inoculation technique was used to assess the resistance of genotypes. An isolate of *P. megakarya* was obtained from a naturally infected pod from the Nkolbisson station. Whole detached leaves from 1 or 2-month-old plants were washed thoroughly with tap water and sterilised with 70% ethanol for 30 s. The experimental design consisted of three replicate plants with six leaves per plant. The inner surface of leaves was scarified along the midrib and inoculated by placing a mycelial disc (6 mm) of *P. megakarya* obtained from a 7-day-old potato dextrose agar (PDA) culture onto each leaf and incubating the samples at 25–26°C in the dark in a humid chamber. Control leaves were inoculated with a sterile agar disc. Necrotic lesions appeared 2–3 days after inoculation and the size of these lesions was measured daily for 6 days.

To determine the effects of sex of the parents on the transmission of resistance, heterosis was estimated (Cilas 1991). Comparative analysis was conducted between parents and their progenies using lesion size.

Phenolic analysis

For the analysis of phenolic compounds, samples were taken 6 days after inoculation of healthy tissue at ∼2 cm from the lesion. Extraction and quantitative measurement of phenolics were performed as described by Omokolo and Boudjeko (2005). Total phenolic compounds were extracted twice using 0.1 M hydrochloric acid (HCl). One gram of fresh tissue was ground in 3 mL 0.1 M HCl. After 30 min incubation at room temperature, the ground material was centrifuged at 6000*g* for 30 min. The supernatant was decanted and the precipitate re-suspended in 3 mL 0.1 M HCl and incubated at room temperature for 15 min. After the second centrifugation, the supernatant was collected and mixed with the previously collected supernatant to constitute the phenolic extract. The concentration of phenolic compounds was determined spectrophotometrically at 725 nm, according to the method of Marigo (1973), using the Folin–Ciocalteu reagent. Phenolic contents were expressed in mg equivalent of chlorogenic acid per g of fresh weight (FW). Qualitative analysis of phenolics was carried out as described by El Hassni *et al*. (2004). Briefly, frozen tissues from different treatments were extracted three times with 80% aq. methanol (MeOH) at 4°C with continuous stirring. The homogenate was centrifuged at 7000*g* for 3 min and the supernatants were stored at −20°C until they were analysed by high performance liquid chromatography (HPLC) using a Waters 600E HPLC (Waters, Milford, MA) equipped with a Waters 990 photodiode Array Detector and Millipore Software for data analysis. An efficient gradient of acetonitrile-*o*-phosphoric acidified bidistilled water (pH 2.6) was used with an Interchrom C18, 5-µm reversed phase column. Phenolics were identified on the basis of their retention time and their spectra in comparison with standards (*p*-coumaric acid, caffeic acid, synapic acid, catechin and epicatechin, luteolin-7-glucoside and apeginin from Sigma-Aldrich, France). When necessary, standards were co-injected to confirm the identity of certain compounds.

The content of hydroxycinnamic acid derivatives, apeginin derivatives and luteolin derivatives were estimated by considering the area of the peak of compounds with the same characteristics. Derived luteolin content was estimated by considering the area of the peak of a compound with the same spectrum as luteolin.

Statistical analyses

Experiments were repeated at least three times. Data presented here are the means ± s.e. of at least three independent

experiments. Analysis of variance (ANOVA) and Newman Student and Keuls tests were used to compare the susceptibility level of better progenies resulting from different crosses and to assess hybrid vigour (Begun and Gabriel 1981). Hierarchical classification of parents and their progenies was obtained by using principal component analysis (PCA). ANOVA and PCA were performed using the statistical program SAS (Anonymous 1997).

Results

Leaf lesions developed 2–3 days after inoculation in both parent clones and their hybrids. In the F16 family, the development of this symptom was less important in the F1612, F1627 and F1614 hybrids (Table 1). In the F3 family, the size of the lesion was important in SNK10, F322, F335, F315, F317 and, more importantly, in the F318 hybrid (Table 1). Six days after inoculation, the mean lesion length was 6.88 ± 0.52 and 9.01 ± 0.71 cm in the SNK10 clone and the F318 hybrid, respectively, with the latter forming significantly ($P < 0.05$) larger lesions than any other hybrid or clone. Two days after inoculation, 87.5% of F16 hybrids showed a negative heterosis. These hybrids were F1614, F1621, F1607, F1608, F1620, F1612 and F1627 (Table 2). The hybrid F1606 showed a positive heterosis, with a value close to 0. Six days after inoculation, 37.5% of F16 hybrids had a negative heterosis. These were the F1614, F1612 and F1627 hybrids (Table 2). For the F3 family, 85.7% of individuals showed a positive heterosis 2 days after inoculation (Table 2).

In healthy leaves, the total phenolic content was higher in F1612 (1.7 mg/g FW), F315 (1.34 mg/g FW), SNK413 (1.39 mg/g FW) and SNK10 (1.31 mg/g FW) (Fig. 1*a*, *b*) individuals. When leaves were wounded or inoculated, total phenolic content increased and varied from 23 to 119% in the F16 family and from 24 to 134% in the F3 family (Fig. 1*a*, *b*). Statistical analysis showed a negative and significant correlation ($r = -0.749$, $P < 0.05$) between the size of the lesions and total phenolics in the F16 family. Direct hierarchical classification of soluble total phenol content in healthy, wounded and inoculated leaves differentiated the hybrids into three groups for the F16 family (Fig. 2*a*) and two groups for the F3 family (Fig. 2*b*). In the F16 family, the first group consisted of all hybrids except F1614 and F1612. The second group consisted of the two parental SNK10 and SNK413 clones and the F1614 hybrid. The third group consisted of the F1612 hybrid, characterised by higher phenolic contents (1.7 mg/g of FW). In the F3 family, all hybrids except F315 constituted the first group and were characterised by lower phenolic contents than the parents (SNK413 and SNK10). The second group contained the two clonal parents and the F315 hybrid.

Qualitative analysis of phenolics in healthy, wounded and inoculated leaves of *T. cacao* (Fig. 3; Table 3) showed the diversity of the compounds present. They were largely represented by hydroxycinnamic acid derivatives and flavonoids. Apigenin was the major compound and represented ~50% of the soluble phenolics in cocoa leaves irrespective of whether the leaves were healthy, wounded or inoculated (Table 3). Some compounds like compound A of the SNK413 (Fig. 3*a*, *b*) clone and compounds B (B1, B2 and B3) (Fig. 3*a*, *b*; Table 3) were stimulated after wounding or inoculation. Compound A was characterised as a hydroxycinnamic acid derivative and was present only in the SNK413 clone (Table 3). The B compounds were apparently luteolin derivatives and were present in the SNK413 and SNK10 clone and in the F1614 hybrid (Fig. 3; Table 3). The content of luteolin derivatives increased with stress

Table 1. Average lesion size (cm) on the midrib of *Theobroma cacao* leaves from hybrids derived from F16 and F3 families

Means within each column followed by the same letter are not significantly different ($P < 0.05$)

Genotypes	Days				
	2	3	4	5	6
F16					
SNK413	1.25 ± 0.26cd	2.55 ± 0.28bc	3.57 ± 0.36abc	3.97 ± 0.48a	4.61 ± 0.30a
SNK10	1.51 ± 0.46de	2.75 ± 0.55c	4.13 ± 0.68c	5.13 ± 0.86b	6.88 ± 0.52c
F1606	1.83 ± 0.30e	3.54 ± 0.55d	5.28 ± 0.61d	7.27 ± 0.69cd	8.79 ± 0.68f
F1612	1.18 ± 0.2cd	2.17 ± 0.53ab	3.12 ± 0.29a	3.94 ± 0.35a	4.59 ± 0.38a
F1627	1.02 ± 0.31bc	1.75 ± 0.56a	3.54 ± 0.69ab	4.15 ± 0.53a	4.77 ± 0.46a
F1607	0a	2.91 ± 0.32c	5.21 ± 0.70d	6.84 ± 0.63cd	7.50 ± 0.82d
F1608	0a	4.01 ± 0.57d	5.99 ± 0.36e	7.51 ± 0.87e	8.44 ± 0.82e
F1620	0a	3.71 ± 0.46d	4.73 ± 0.81d	6.30 ± 0.92c	7.83 ± 0.89de
F1614	1.36 ± 0.24cd	2.09 ± 0.53ab	3.29 ± 0.71ab	3.70 ± 0.71a	4.77 ± 0.82a
F1621	0.76 ± 0.83b	2.55 ± 0.65bc	3.77 ± 0.53bc	4.94 ± 0.59b	5.96 ± 0.44b
F3					
SNK413	1.25 ± 0.26cd	2.55 ± 0.28c	3.57 ± 0.36b	3.97 ± 0.48a	4.61 ± 0.30a
SNK10	1.51 ± 0.46d	2.75 ± 0.55cd	4.13 ± 0.68bc	5.13 ± 0.86bc	6.88 ± 0.52c
F311	0.97 ± 0.20b	2.83 ± 0.40cde	3.93 ± 0.4bc	4.78 ± 0.68b	5.35 ± 0.85b
F318	0a	3.50 ± 0.36f	5.55 ± 0.33ef	7.38 ± 0.53e	9.01 ± 0.71d
F322	1.5 ± 0.17d	3.10 ± 0.11def	4.48 ± 0.16cd	5.3 ± 0.45bc	6.94 ± 0.36c
F335	1.17 ± 0.47bc	1.94 ± 0.37b	4.02 ± 0.85bc	5.67 ± 0.85c	6.71 ± 0.87c
F327	0a	0a	2.43 ± 0.67a	3.54 ± 0.70a	4.58 ± 0.80a
F315	0a	3.25 ± 0.65ef	4.96 ± 0.41de	6.51 ± 0.31d	7.38 ± 0.47c
F317	0a	3.38 ± 0.13f	5.78 ± 0.52f	6.41 ± 0.45d	6.87 ± 0.56c

Table 2. Heterosis values (%) by comparison of lesion necrotic size between parents and their progenies

Genotypes	Days				
	2	3	4	5	6
F16					
F1614	−2.16	−21.13	−14.54	−18.68	−16.90
F1621	−45.32	−3.77	−2.07	+8.57	+3.83
F1607	−100	+9.81	+35.32	+50.32	+30.66
F1608	−100	+51.32	+55.58	+65.05	+47.04
F1620	−100	+40	+22.86	+38.46	+36.41
F1606	+0.31	+33.58	+37.14	+58.90	+53.13
F1612	−15.11	−18.11	−18.96	−13.40	−20.03
F1627	−26.62	−34	−8.05	−8.79	−16.89
F3					
F318	−100	+32	+44.15	+62.19	+57
F311	−30.21	+7	+2	+5	−7
F322	+7.90	+17	+16.36	+16.48	+21
F335	−15.80	−26.79	+4.42	+25	+21
F327	−100	−100	−36.88	−22.20	−20.20
F315	−100	+22.64	28.83	+43	+16.90
F317	−100	+27.55	+28.83	+43.07	+19.68

and the increase was more pronounced in inoculated leaves (Table 3). In addition to the enhancement of phenolic compound accumulation, an unidentified hydroxicinnamic acid derivative, compound X, has been detected only in inoculated leaves of the tolerant SNK413 clone (Fig. 3*b*).

Discussion

The main goal of this study was to analyse the heritability of resistance to *P. megakarya* in cocoa by the evaluation of the necrotic area and phenolic compounds in healthy, wounded and inoculated leaves. Because of the performance recorded in their progeny (heritability), the most resistant progeny could be grown to assess other useful characters, such as yield. Our observations showed that wound inoculation of 1- or 2-month-old leaves of cocoa derived from the genotypes SNK413 and SNK10, develop necrosis of the main vein. The size of necrotic lesions was higher in the SNK10 than in the SNK413 clone. Similar results were obtained by Djocgoue *et al*. (2006) when cocoa leaves of the same clone were inoculated with *P. megakaya*. Some hybrids from the F16 family (F1612, F1614 and F1627) were characterised by localised lesions. The hybrids produced from the two clones were as tolerant to *P. megakarya* as the best parent, SNK413, and should inherit that tolerant character from this parent. There was no significant difference between genotypes coming from reciprocal crossbreeding of ♀SNK413 × ♂SNK10 and ♀SNK10 × ♂SNK413 when the lesion size on the main vein was evaluated. The heterosis effect of each F16 and F3 family when comparing the development of necrosis revealed a higher variability within both families. In fact, 62.5% of F16 hybrids and 71% of F3 hybrids presented a positive heterosis 6 days after inoculation, a testament to the hybrid vigour manifestation within these two families. Hybrids that present a positive heterosis for a character might have genes containing additive effects in some situations that have an important implication in the transmission of that character (Cilas *et al*. 1998).

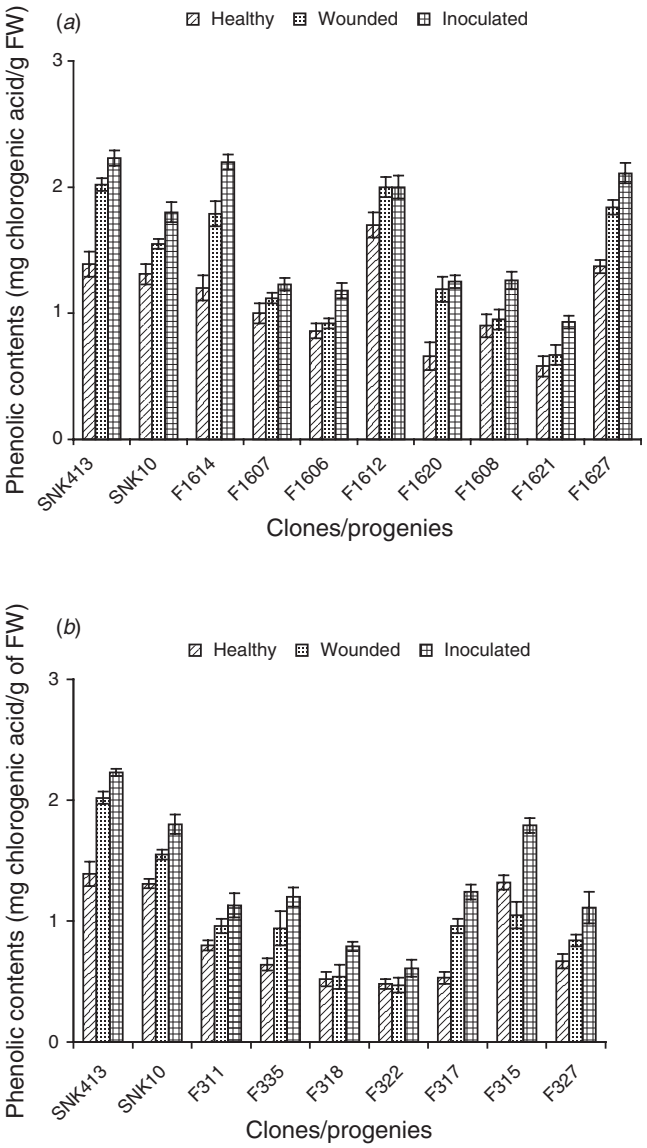

Fig. 1. Total soluble phenolic content (µg/mg fresh weight) in healthy, wounded and *Phytophthora megakarya*-inoculated leaves of (*a*) F16 and (*b*) F3 hybrid families of *Theobroma cacao.*

In the healthy plants, the highest phenolic content was observed in the F1612, F1614 hybrids and the SNK413 clone, all of which developed localised and restricted lesions when inoculated with the pathogen. After inoculation, an increase in the soluble phenolic content was observed in both parents and all of their hybrids. This observation has also been made on cocoa pods inoculated with *P. megakarya* (Nguefack 1994; Omokolo *et al*. 2002). A negative heterosis effect was determined in almost all plants except the F1612 and F1614 hybrids, which showed an additive effect and increased vigour. Generally, the accumulation of phenolics following infection by pathogen involves the neosynthesis of specific phenol compounds (Tan *et al*. 2004; Conceiçao *et al*. 2006). In the *T. cacao* × *P. megakarya* interaction, qualitative analysis of phenolics in leaves by HPLC

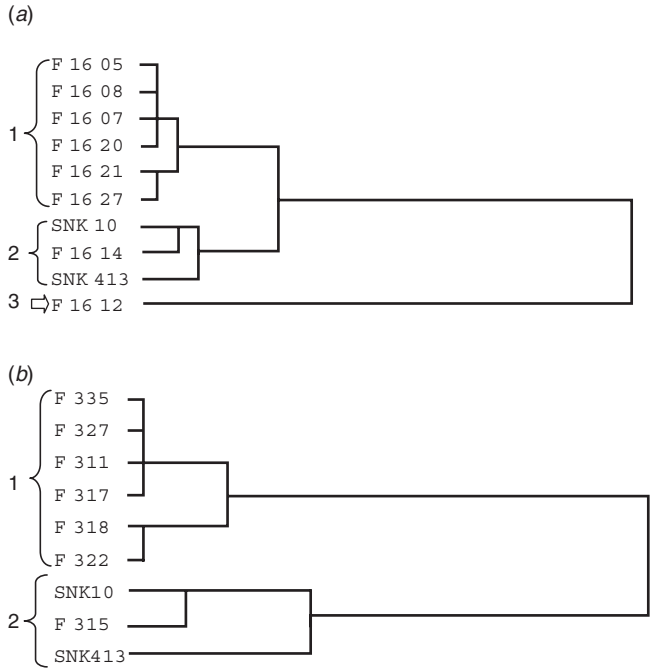

Fig. 2. Direct hierarchical classification obtained with soluble total phenols of leaves of SNK413, SNK10 clones and hybrids from (*a*) F16 and (*b*) F3 hybrid families of *Theobroma cacao* structuring the F16 hybrid family into three groups and the F3 hybrid family into two groups, respectively.

showed a higher accumulation of some luteolin derivatives (flavonones), a compound A (hydroxycinnamic acid derivative) and an unidentified compound X. The stimulation of the flavonone (luteolin) compound might be due to the activation of the jasmonic acid pathway during the development of the defence reaction of cocoa against *P. megakarya*. Recent research has shown that treatment of *Hypericum perforatum* cells with jasmonic acid before inoculation with *Colletotrichum gloeosporioides* led to selective accumulation of flavonones (Conceiçao *et al*. 2006).

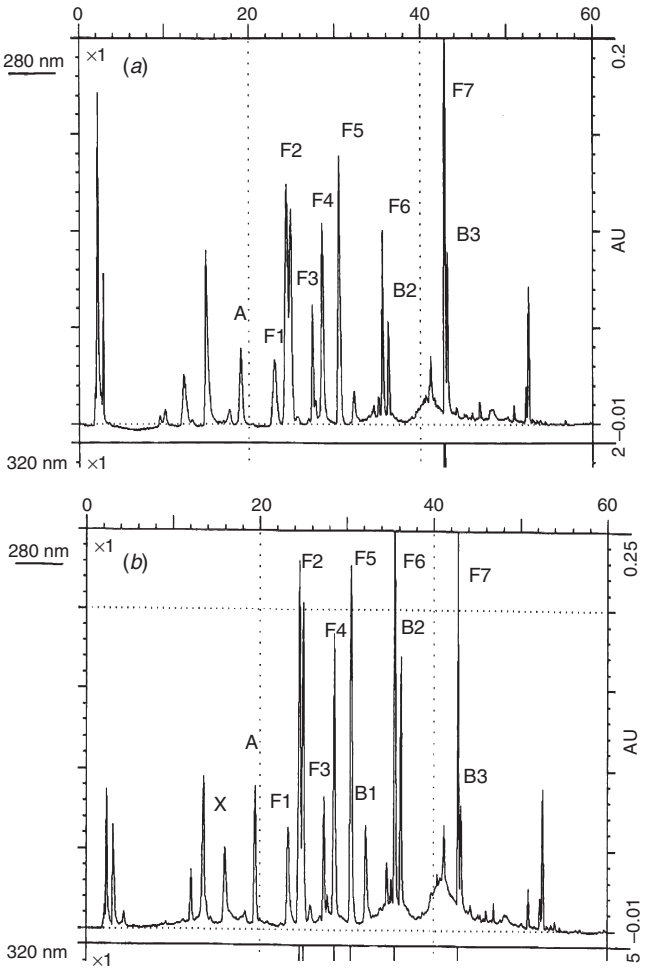

Fig. 3. HPLC chromatogram of phenolic compounds in *Theobroma cacao* leaves of SNK413 which were (*a*) healthy or (*b*) inoculated with *Phytophthora megakarya*, showing (1) the dominance of apeginine (F1, F2, F, F4, F5, F6, F7); (2) an increase of luteolin derivatives 'compounds B' in infected leaves; (3) the appearance of compound 'X' in infected leaves.

Table 3. The quantity of various phenol derivatives (μg/mg fresh weight) in healthy, wounded and *Phytophthora megakarya*-inoculated leaves of SNK413 and SNK10 clones and F317 and F1614 hybrids

Genotypes	Status	Luteolin derivatives	Apeginin derivatives	Hydroxycinnamic acids derivatives	Compound A	Compound X
SNK413	Healthy	0.34 ± 0.07	0.57 ± 0.10	0.12 ± 0.08	0.04 ± 0.01	0
	Wounded	0.46 ± 0.04	0.98 ± 0.05	0.10 ± 0.04	0.05 ± 0.02	0
	Inoculated	0.97 ± 0.06	0.96 ± 0.05	0.10 ± 0.06	0.09 ± 0.03	0.08 ± 0.01
SNK10	Healthy	0.11 ± 0.03	0.67 ± 0.01	0.31 ± 0.04	0.05 ± 0.01	0
	Wounded	0	0.64 ± 0.04	0.29 ± 0.03	0.05 ± 0.02	0
	Inoculated	0	0.65 ± 0.03	0.17 ± 0.03	0.06 ± 0.02	0
F1614	Healthy	0.09 ± 0.03	0.75 ± 0.08	0.14 ± 0.07	0	0
	Wounded	0.30 ± 0.05	0.76 ± 0.09	0.12 ± 0.02	0	0
	Inoculated	0.60 ± 0.06	0.82 ± 0.08	0.15 ± 0.03	0	0
F317	Healthy	0	0.30 ± 0.07	0.16 ± 0.04	0	0
	Wounded	0	0.23 ± 0.07	0.12 ± 0.06	0	0
	Inoculated	0	0.46 ± 0.09	0.25 ± 0.05	0	0

In conclusion, some plants of the F16 family (F1612, F1614 and F1627) obtained by ♀SNK413 × ♂SNK10 crossbreeding acquire necrotic lesions similar to the SNK413 clone when inoculated with *P. megakarya*. These hybrids received disease tolerance characteristics from the tolerant clone SNK413. In addition, qualitative analysis of phenolic compounds from the parents and two hybrids obtained after reciprocal crossbreeding showed an accumulation of luteolin only in the SNK413 clone and in a hybrid whose female parent was the SNK413. This suggests that some resistance characters in cocoa to *Phythophthora* spp. are cytoplasmic. Further analysis of some hybrids of the same group will be carried out to confirm this observation.

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References

Anonymous (1997) 'SAS/STAT user's guide. Release 612.' (SAS institute Inc.: Cary, NC)

Begun JM, Gabriel KR (1981) Closure of the Newman-Keuls multiple comparisons procedure. *Journal of American Statistics Association* **76**, 241–245. doi: 10.2307/2287817

Cilas C (1991) Estimation of some genetics parameters of different crosses in plants of cocoa. *Cafe, Cacao, The* **25**, 3–13.

Cilas C, Lanaud C, Paulin D, Nyasse S, Ngoran JA, Kebe BI, Ducamp M, Flamant MH, Risterucci AM, Pieretti, Saunigo O, Thevenon JM, Despraux D (1998) La résistance à la pourriture des cabosses due à Phytophthora spp. Recherche des composantes de la résistance. *Plantation, Recherche, Dévelopement* **5**, 441–445.

Conceiçao LFR, Ferreres F, Tavares RM, Dias ACP (2006) Induction of phenolic compounds in *Hypericum perforatum* L.; cells by *Colletotrichum gloeosporioides* elicitations. *Phytochemistry* **67**, 149–155. doi: 10.1016/j.phytochem.2005.10.017

Daayf F, Schimtt A, Belanger RR (1997) Evidence of phytoalexins in cucumber leaves infected with powdery mildew following treatment with leaf extracts of *Reynoutria sachalinensis*. *Plant Physiology* **113**, 719–727.

Despréaux D, Clément D, Partiot M (1989) La pourriture brune des cabosses du cacaoyer au Cameroun: mise en évidence d'une caractère de résistance au champ. *Agronomie* **9**, 683–691.

Djocgoue PF (1998) Analyse des variations biochimiques liées au dévelopement et l'infection par *Phytophthora megakarya* Bras. et Grif. chez *Theobroma cacao* L. PhD thesis, Université de Yaoundé, Cameroon.

Djocgoue PF, Boudjeko T, Nankeu DJ, Efombagn MIB, Nyasse S, Omokolo DN (2006) Comparative assessment of the resistance of Cocoa (*Theobroma cacao* L.) progenies from SNK10 × SNK413; ICS84 × ICS95 to *Phytophthora megakarya* in Cameroon by measuring size of necrotic lesion along the midrib. *Plant Pathology Journal* **5**, 329–335.

El Hadrami I, Ramos T, El Bellaj M, El Idrissi-Tourane A, Macheix JJ (1997) A sinapic derivative as induced defence compound of date palm against *Fusarium oxysporum* f. sp. *albedinis*, the agent causing bayoud disease. *Journal of Phytopathology* **145**, 329–333.

El Hassni M, J'aiti F, Dihazi A, Ait Barka E, Daayf F, El Hadrami I (2004) Enhancement of defense response against Bayoud disease by treatment of date palm seedling with hypoagressive *Fusarium oxysporum* isolate. *Journal of Phytopathology* **152**, 182–189. doi: 10.1111/j.1439-0434.2004.00824.x

Gallais A (1990) 'Théorie de la sélection en amélioration des plantes.' (Masson: Paris)

Gray A (2000) The world cocoa market outlook. Ghana Conference Paper, LMC International Press. Available at http://www.treecrops.org/crops/cocoaoutlook.pdf (verified 26 October 2006)

Kodama O, Miyakawa J, Akatsuka T, Kiyosawa S (1992) Sakuranetin, a flavanone phytoalexin from ultra-violet irradiated rice leaves. *Phytochemistry* **31**, 3807–3809. doi: 10.1016/S0031-9422(00)97532-0

Marigo G (1973) Méthode de fractionnement et d'estimation des composés phénoliques chez les végétaux. *Analysis* **2**, 106–110.

Ndoumbe-Nkeng M, Bieyssé D, Cilas C (2001) Multi-trait selection in a diallel crossing scheme of cocoa. *Plant Breeding* **120**, 365–367. doi: 10.1046/j.1439-0523.2001.00590.x

Nguefack J (1994) Etude des relations hôte-parasite dans l'interaction compatible entre *Theobroma cacao* L. et *Phytophthora megakarya* (Nature, localisation et implication des phénols du cortex du fruit dans la résistance du cacaoyer). PhD thesis, Université de Yaoundé, Cameroon.

Nyassé S (1997) Etude de la diversité de *Phytophthora megakarya* et caractérisation de la résistance du cacaoyer (*Theobroma cacao* L.) à ce pathogène. PhD thesis, Institut National Polytechnique de Toulouse, France.

Nyassé S, Cilas C, Herail C, Blaha G (1995) Leaf inoculations as an early screening test for cocoa (*Theobroma cacao* L.) resistance to *Phytophthora* black pod disease. *Crop Protection* **14**, 657–663. doi: 10.1016/0261-2194(95)00054-2

Omokolo ND, Boudjeko T (2005) Comparative analyses of alteration in carbohydrates, amino acids, phenols and lignin in roots of three cultivars of *Xanthosoma sagittifolium* (macabo) infected by *Pythium myriotylum*. *South African Journal of Botany* **71**, 432–440.

Omokolo ND, Nankeu DJ, Niemenak N, Djocgoue PF (2002) Analysis of aminoacids and carbohydrates in the cortex of nine clones of *Theobroma cacao* L. in relation to their susceptibility to *Phytophthora megakarya* Bra. and Grif. *Crop Protection* **21**, 395–402. doi: 10.1016/S0261-2194(01)00121-1

Omokolo ND, Nankeu DJ, Niemenak N, Boudjeko T (2003) Variation of β-1,3-glucanase, chitinase and polyphenol oxidase activities in cacao pods upon *Phytophthora megakarya* inoculation. *African Crop Science Journal* **11**, 97–106.

Tan J, Schneider B, Svatos A, Bednarek P, Liu J, Hahlbrock K (2004) Universally occurring phenylpropanoid and species-specific indolic metabolites in infected and uninfected *Arabidopsis thaliana* roots and leaves. *Phytochemistry* **65**, 691–699. doi: 10.1016/j.phytochem.2003.12.009

Treuter D, Feucht W (1990) The pattern of flavan-3-ols in relation to scab resistance of apple cultivars. *Journal of Horticultural Science* **65**, 511–517.

Wood GA, Lass RA (1985) 'Cocoa, tropical agriculture series.' 4th edn. (Longman: London)

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