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Phenolic content and heritability of resistance in four hybrid populations of *Theobroma cacao* L. after leaves inoculation with *Phytophthora megakarya* Bras. et Grif.

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ABSTRACT

Cocoa is cultivated in Cameroon for its broad beans. The commercialization of cocoa seeds constitutes a major source of income to farmers. Nevertheless, cacao black pod disease caused by *Phytophthora megakarya* is responsible of about 80% of cocoa production loss in Cameroon without any protection method. To assess the resistance of cocoa plants against this pathogen, necrotic lesions and phenolic content were conducted on 3 clones (SNK16, ICS40, Sca12) and their progenies (families F40, F12, F20 and F25) after leaves inoculation. The existence of strong hybrid vigour has been shown. All hybrid genotypes manifested a positive heterosis effect for this symptom suggesting the existence of hybrid vigour. Some hybrids like F40.6, F40.7, F40.8, F40.9, F40.10, F12.10, F12.15, F20.7, F20.10, F25.2, F25.5 and F25.7 were characterized by localized lesions. A negative correlation between the size of necrotic lesions and the total phenolic compound was demonstrated. Three genotypes of the F40 family (F40.8, F40.9 and F40.13), one of the F12 (F12.15) and two of the F25 (F25.2 and F25.8) had small lesions and high concentrations of phenols. These six genotypes can be considered as elite clones with high tolerance to *P. megakarya*. The values of the heritability of lesion size and the total phenolic content in offsprings don't show the maternal effect.

Keywords: Cocoa, *Phytophthora megakarya*, heterosis, heritability, necrosis, phenols.

INTRODUCTION

Cocoa is a tropical forest plant with its diversity centre located in South America (Bailey et al., 2005). It was introduced in Cameroon in 1892 by the Germans and is cultivated mainly for its seeds (cocoa beans) (Nya Ngatchou, 1979). Cocoa (*Theobroma cacao* L.) provides a substantial income for small holders in the tropics. In Cameroon, the production has increased from 145,000 tons in

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2004 to 230,000 tons in 2013 and this country remains the fifth world cocoa producer (ICCO, 2013). A strong correlation exists between the production and the use of hybrids. In fact, the main producing countries are those who use a high percentage of hybrids in their farms (Indonesia 76%, Ivory Coast 69.2%, Malaysia 69%, Ghana 63.2%, Cameroon 25.1% and Ecuador 17.4%. In Cameroon, most of the cultivated cocoa trees were originally derived from old varieties introduced by German colonialists, 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, environmental adaptation and vigour, these hybrids have been exploited on a large scale. These hybrids varieties have also shown large phenotypic variation for many traits and are not readily accepted by farmers (Ndoumbe-Nkeng et al., 2001).

Africa alone contributes for about 67% of world cocoa production (Anonymous, 2002). This production is seriously affected by pod rot disease caused by various species of *Phytophthora*. Consequently, crop losses are estimated at 30% of the world production (Wood and Lass, 2001). In Cameroon, this disease has particular importance due to the existence of a single species (*Phytophthora megakarya*), which is considered as the most aggressive in the field (Nyasse et al., 1997; Omokolo et al., 2002).

Resistance to *P. megakarya* infection has become a major breeding target. The methods available for controlling cocoa diseases are fungicide application, use of resistant cultivars and other appropriate cultural practices. An increase in the effectiveness of control can be expected when these methods are combined. However, chemical control is expensive, commercially non-viable, and environmentally harmful.

Cocoa breeders continue to face the problem of high heterogeneity between individuals derived from one cross and heterogeneous transmission of genetic traits to the progeny. The hybrid vigour is manifested by increased size, growth rate and/or other parameters resulting from the increase in heterozygosity in the F1 generation following crosses between inbred lines (Gallais, 1990). Nyasse (1997); Djocgoue (1998) and Nyasse et al. (2002) showed that there is a correlation between the resistance of cocoa to *P. megakarya* and the size of the necrotic lesion following artificial inoculation to screen pods and leaves for disease development.

Plants generate some biochemical and physiological reactions when they are faced with biotic or abiotic stress factors, and several chemical compounds are synthesized as a consequence. Defence reactions may develop several hours or a few days after stimulation (Desender et al., 2007). Plants have developed appropriate defence mechanisms to recognize and resist inevitable pathogen attacks. Plants use inherent physical and chemical barriers to effectively stop a pathogen invasion, and their inducible defence reactions are activated by pathogen attacks (Aktaş and Guven, 2005).

Research to date has demonstrated that phenolic compounds assist in strengthening the cell walls against pathogens and inhibiting fungal growth. Changes in the amount of phenolic compounds in plants are indicators of susceptibility to disease (Yao et al., 1995).

It is known that various cell wall compounds inhibit the activation of cell wall-Enzymes secreted by fungal pathogens during the infection are involved in the decomposition of the cell decomposing fungal enzymes. For example, phenolics and proteins bound to the cell wall inhibit fungal enzymes. In fact, decomposition and strengthening of the cell wall are considered to be the key events in fungal pathogen infection (Vidhyasekaran, 2007).

In this study, early screening tests have been applied on nursery material in order to evaluate the resistance level of Cocoa (*Theobroma cacao* L.) progenies from SNK10

x SNK413; ICS84 x ICS95 to *Phytophthora megakarya* in Cameroon.

MATERIALS AND METHODS

Cocoa plant material

Three cocoa clones with different sensibility to *P. megakarya* available in gene banks of the Cameroon Cocoa Development Corporation (SODECAO) at Mengang Station (Southern Cameroon) were used to create four progenies: one local Trinitario (SNK16, Tolerant to *P. megakarya*), one Trinitario introduced from Trinidad (ICS40, high sensibility to *P. megakarya*) and one Forestero (Sca12, Tolerant to *P. megakarya*). Crossings were realized in Mengang Station of SODECAO in May, June and July 2012 using hand-pollinisation techniques (Cilas, 1991). The four progenies obtained were:

F_{40} : (♀) ICS_{40}× (♂) SCa_{12}
F_{12} : (♀) SCa_{12} × (♂) ICS_{40}
F_{20} : (♀) ICS_{40}× (♂) SNK16
F_{25} : (♀) SNK16× (♂) ICS_{40}

Production of seedlings and grafts

Seeds from pods harvested in experimental field were sown in the nursery at Cameroon Cocoa Development Corporation (SODECAO) at Mengang Station (Cameroon) and 371 hybrids plants were obtained. Parental plantlets were obtained through top-grafting by using bud wood from the three clones listed above. This grafting was done on non-specific young cocoa plantlets.

Leaf inoculation and analysis

The leaf test is an artificial inoculation method that can be used to assess the resistance of genotypes. Briefly, whole detached leaves from one or three month's old plants were washed thoroughly with tap water and sterilized with ethanol 70% for 30 s. The experimental design consisted of three replications of four leaves per seedling. The inner surface of leaves were scarified along the midrib and inoculated by deposition of a mycelium disc (6 mm) of pure culture of *Phytophthora megakarya* obtained after 7-days pure culture grown in PDA medium. The inoculated leaves were incubated in humid chamber at 25-26 °C in total darkness. Control leaves were inoculated with sterile agar disc in the same conditions. The isolate used of *Phytophthora megakarya* belonged to « Phy-» strain and were provided by the Institute of Agricultural Research for Development (IRAD), Nkolbisson research station. Two days after incubation, the size of the lesion developed on the inoculated leaves was measured every two days until day 6.

In order to emphasize the effects of the parent sex on transmission of resistance, two genetic parameters were estimated: the heterosis (Zahour, 1992) and the heritability. Heritability (h^2) was estimated according to Falconer (1974). Heritability was obtained by slope of the regression line between parents and their progenies using length of necrosis.

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Total phenolic content

The total phenolic content was determined following the method of Singleton and Rossi (1965). A sample (50 mg) was extracted with 1 ml of 70% aqueous ethanol at room temperature. The mixture was centrifuged at 1000g for 15 min. The supernatant (200 µl) was mixed with 1.5 ml of Folin–Ciocalteu reagent, and allowed to stand at room temperature for 5 min; then 1.5 ml of sodium bicarbonate solution (0.566 M) was added to the mixture. After 60 min, absorbance was read at 725 nm. Results were expressed as gallic acid equivalents. The concentration used was in a range between 0.02 and 0.1 mg/ml.

Statistical analyses

Data presented are the means ± SE of at least three independent experiments. ANOVA and Tukey test permitted to analyse and compare the susceptibility level of better progenies resulting from different crosses and

to assess hybrid vigour (Begun and Gabriel, 1981). Cluster analyses of parents and their progenies based on the necrotic size of the lesion along the midrib from day 2 to 6, using the Unweighted Pair Group Method with Arithmetic Average (UPGMA) on the basis of Nei's (1978) genetic distance were performed with the assistance of SPSS 17.0 for windows.

RESULTS

Hand-pollinisation

Hand-pollinisation test was less successful in F25 and F20 families with 4 and 2% respectively. These results were better in F40 (36%) and F12 (46%) families (Table 1).

Evolution of the lesion

On leaves wounded and inoculated with agar disc containing *P. megakarya* mycelium, the development of the necrosis appeared 2-3days after inoculation in both parent clones and their hybrids.

Generally, in the F40 family, the size of the lesions varied between 0.7 cm (day 2) and 6.43 cm (day 6). At day 2, the development of lesions was less important in the F40.06 and F40.09 hybrids. Six days after inoculation, the mean lesion length was 1.43 ± 0.05 and 6.43 ± 0.00 cm in the F40.08 hybrid and the ICS 40 clone respectively with the latter forming significantly ($P < 0.05$) larger lesions than any other clone or hybrid (Table 2).

In the F12 family, Back-cross of the F40 one, F12.10 and F12.15 hybrids displayed the lower development of this symptom at day 6 with an average of 1.10 ± 0.10 cm (Table 3).

The larger lesions in F20 and F25 families were observed in F20.06 and F20.09 hybrids, and in F25.09 and F25.03 hybrids in the other part. But their values remained lower as compared to the value of their ICS40 parents (Tables 4 and 5).

At 5% degree of heterogeneity, direct hierarchical classification of necrotic lesion size in inoculated leaves at days two, four and six differentiated into three groups for the F40 and F12 families (Figure 1) respectively. The first group of the F40 family contained 11 individuals characterised by small lesion size while the second group, with two hybrids, displayed average lesion size. The last one is composed by F40.05 hybrid and the two parents and presented larger lesion necrotic size. In the F12 family, the first group more polymorphic, consisted of 14 individuals with low necrotic lesion size in inoculated leaves. The second group, with two individuals (SCA12 parent and F12.14 hybrid), is characterised by average lesion size. The third group consisted of ICS40 parent alone, displayed a great necrotic lesion. Dendrograms of F20 and F25 families presented the same structure with two groups each where ICS40 clone formed alone the second group with great necrotic lesion size (Figures 2a and 2b).

Heterosis

Except the hybrid F40.05 that showed a negative heterosis, all hybrids of the four families studied displayed a positive heterosis even two and four days after infection. These hybrids were therefore more vigorous than their parents (Tables 6 and 7).

Heritability

The values of the heritability (h^2) have been determined according to the size of lesion. Concerning this character, values obtained are 0.48 and 0.45, respectively, for F40 [(♀) ICS_{40}× (♂) SCa_{12}] and F12 [(♀) SCa_{12} × (♂) ICS_{40})] crosses. In F20 and his reciprocal crossings F25, the value of heritability was respectively 0.65 and 0.47 (Table 8).

Variation of the phenolic contents

In healthy leaves, the total phenolic content was higher in F40.09 (7.95 mg/g of FW) and F40.13 (7.67 mg/g of FW) individuals (Figure 3). When leaves were wounded or inoculated, total phenolic

contents increased and varied from 18 to 63% in the F40 family and from 10to 304% in the F12 family. For the F20 family, this metabolite varied from 100 to 128% and 51 to 105% in the F25 family. But this evolution remained less important (22 to 49%) in the ICS 40 clone contrary to two other clones (Sca12, 129 to 231% and SNK16, 208 to 213%) (Figure 4).

In our trials, a significant negative correlation (r = -0,692 for F30 family and r = -0.598 for F12 family one part and r = -0.675 for F20 family and r = -0.430 for F25 family in the other part) at $P < 0.01$ is noticed between the necrosis length and the phenolic contents (data not shown).

Table 1: Rate of success of hand-pollinisation.

Families	Crossing	Number of test	Number of success	Percentage of success
F40	(♀)ICS_{40}× (♂) SCa_{12}	92	33	36,06%
F12	(♀) SCa_{12} × (♂) ICS_{40}	104	48	46,15%
F25	(♀)ICS_{40} × (♂) SNK_{16}	119	5	4,20%
F20	(♀)SNK_{16} × (♂) ICS_{40}	85	2	2,43%

Table 2: Average lesion size (cm) on the midrib of *Theobroma cacao* leaves from hybrids derived from F40 family.

	Average lesion size (cm)		
Genotypes	**Day 2**	**Day 4**	**Day 6**
Parents			
ICS_{40}(♀)	1.91 ± 0.00^{h}	3.41 ± 0.00^{f}	6.43 ± 0.00^{g}
SCa_{12} (♂)	1.42 ± 0.00^{g}	3.45 ± 0.00^{f}	4.90 ± 0.00^{f}
F40			
F40.01	0.80 ± 0.10^{bc}	1.20 ± 0.26^{ab}	1.97 ± 0.29^{abc}
F40.02	0.90 ± 0.35^{bcde}	1.60 ± 0.10^{bc}	2.17 ± 0.67^{abc}
F40.03	1.20 ± 0.20^{fg}	2.60 ± 0.66^{e}	4.03 ± 0.90^{e}
F40.04	1.10 ± 0.17^{def}	2.37 ± 0.47^{de}	3.30 ± 0.36^{d}
F40.05	1.10 ± 0.00^{def}	3.50 ± 0.56^{f}	5.97 ± 0.64^{g}
F40.06	0.73 ± 0.15^{b}	1.07 ± 0.12^{ab}	1.93 ± 0.15^{abc}
F40.07	0.90 ± 0.00^{bcde}	0.93 ± 0.06^{a}	1.76 ± 0.30^{ab}
F40.08	0.97 ± 0.06^{bcdef}	1.13 ± 0.12^{ab}	1.43 ± 0.05^{a}
F40.09	0.70 ± 0.10^{b}	1.10 ± 0.10^{ab}	1.83 ± 0.58^{ab}
F40.10	1.03 ± 0.12^{cdef}	1.50 ± 0.20^{bc}	2.03 ± 0.15^{abc}
F40.11	1.07 ± 0.12^{cdef}	1.53 ± 0.15^{bc}	1.80 ± 0.26^{ab}
F40.12	1.13 ± 0.29^{ef}	1.53 ± 0.25^{bc}	2.26 ± 0.15^{bc}
F40.13	0^{a}	2.00 ± 0.30^{cd}	2.63 ± 0.20^{cd}
F40.14	0.83 ± 0.15^{bcd}	2.03 ± 0.15^{cd}	2.66 ± 0.47^{cd}

Values with the same letter in the same column and in the same family are not significant different ($P < 0.05$).

Table 3: Average lesion size (cm) on the midrib of *Theobroma cacao* leaves from hybrids derived from F12 family.

	Average lesion size (cm)		
Genotypes	**Day 2**	**Day 4**	**Day 6**
Parents			
ICS_{40} (♂)	1.91 ± 0.00^{h}	3.41 ± 0.00^{f}	6.43 ± 0.00^{g}
SCa_{12} (♀)	1.42 ± 0.00^{g}	3.45 ± 0.00^{f}	4.90 ± 0.00^{f}
F12			
F12.01	0.77 ± 0.15^{b}	1.83 ± 0.31^{de}	2.40 ± 0.10^{bcd}
F12.02	0.87 ± 0.06^{b}	2.00 ± 0.50^{def}	3.70 ± 0.20^{ef}
F12.03	0.77 ± 0.12^{b}	1.10 ± 0.10^{ab}	1.43 ± 0.15^{ab}
F12.04	1.10 ± 0.00^{c}	2.40 ± 0.20^{f}	2.70 ± 0.10^{cde}
F12.05	0.87 ± 0.12^{bc}	1.90 ± 0.17^{def}	3.43 ± 0.40^{de}
F12.06	0.77 ± 0.23^{b}	1.20 ± 0.26^{abc}	2.33 ± 0.31^{bc}
F12.07	0.67 ± 0.12^{b}	1.47 ± 0.12^{bcd}	2.10 ± 0.17^{abc}
F12.08	0.80 ± 0.10^{b}	1.97 ± 0.38^{def}	2.70 ± 0.40^{cde}
F12.09	1.27 ± 0.20^{de}	1.67 ± 0.35^{cde}	2.53 ± 0.45^{cd}
F12.10	0^{a}	0.70 ± 0.10^{a}	1.10 ± 0.10^{a}
F12.11	0.73 ± 0.15^{b}	1.63 ± 0.32^{bcde}	2.10 ± 0.56^{abc}
F12.12	1.33 ± 0.15^{de}	2.10 ± 0.26^{ef}	2.53 ± 0.25^{cd}
F12.13	0.83 ± 0.21^{b}	1.73 ± 0.68^{cde}	4.53 ± 2.05^{fg}
F12.14	1.47 ± 0.31^{e}	3.70 ± 0.10^{g}	4.80 ± 0.61^{g}
F12.15	0^{a}	0.80 ± 0.26^{a}	1.10 ± 0.10^{a}

Values with the same letter in the same column and in the same family are not significant different ($P < 0.05$).

Table 4: Average lesion size (cm) on the midrib of *Theobroma cacao* leaves of from hybrids derived from F20 family.

	Average lesion size (cm)		
Genotypes	**Day 2**	**Day 4**	**Day 6**
Parents			
ICS_{40} (♀)	1.91 ± 0.00^{e}	3.41 ± 0.00^{d}	6.42 ± 0^{d}
SNK_{16} (♂)	0.88 ± 0^{d}	2.24 ± 0^{bc}	3.09 ± 0^{abc}
F20			
F20.01	0.83 ± 0.15^{d}	2.37 ± 0.67^{c}	3.07 ± 0.76^{abc}
F20.02	0.33 ± 0.32^{ab}	1.53 ± 0.76^{abc}	2.53 ± 0.92^{abc}
F20.03	0.47 ± 0.12^{abc}	1.67 ± 0.58^{abc}	2.43 ± 0.40^{abc}
F20.04	0.70 ± 0.20^{cd}	1.83 ± 0.57^{abc}	2.47 ± 0.76^{abc}
F20.05	0.67 ± 0.31^{cd}	2.07 ± 0.40^{abc}	2.73 ± 0.64^{abc}
F20.06	0.17 ± 0.06^{ab}	2.43 ± 0.35^{c}	3.67 ± 1.07^{c}
F20.07	0.20 ± 0.00^{ab}	1.10 ± 0.36^{a}	1.77 ± 0.06^{a}
F20.08	0.50 ± 0.26^{bc}	1.50 ± 0.89^{abc}	2.27 ± 0.55^{abc}
F20.09	0.40 ± 0.17^{abc}	2.33 ± 0.76^{c}	3.50 ± 1.25^{bc}
F20.10	0.20 ± 0.10^{ab}	1.17 ± 0.29^{a}	1.73 ± 0.68^{a}
F20.11	0.13 ± 0.06^{a}	1.27 ± 0.31^{ab}	2.10 ± 0.46^{ab}
F20.12	0.13 ± 0.23^{a}	2.20 ± 0.50^{bc}	3.43 ± 1.26^{bc}

Values with the same letter in the same column and in the same family are not significant different ($P < 0.05$).

Table 5: Average lesion size (cm) on the midrib of *Theobroma cacao* leaves of from hybrids derived from F25 family.

	Average lesion size (cm)		
Genotypes	**Day 2**	**Day J4**	**Day 6**
Parents			
ICS_{40} (♂)	1.91 ± 0.00^{e}	3.41 ± 0.00^{d}	6.42 ± 0^{d}
SNK_{16} (♀)	0.88 ± 0^{d}	2.24 ± 0^{bc}	3.09 ± 0^{abc}
F25			
F25.01	0.40 ± 0.17^{ab}	1.70 ± 0.53^{bc}	2.30 ± 1.06^{abc}
F25.02	0.43 ± 0.06^{ab}	1.13 ± 0.32^{ab}	1.67 ± 0.83^{ab}
F25.03	0.53 ± 0.21^{abc}	1.63 ± 0.90^{abc}	2.57 ± 1.12^{abc}
F25.04	0.50 ± 0.17^{ab}	1.37 ± 0.32^{ab}	2.30 ± 0.30^{abc}
F25.05	0.57 ± 0.15^{abc}	0.87 ± 0.15^{a}	1.40 ± 0.26^{a}
F25.06	0.67 ± 0.21^{bc}	1.53 ± 0.40^{abc}	2.37 ± 0.32^{abc}
F25.07	0.47 ± 0.21^{ab}	1.33 ± 0.35^{ab}	1.67 ± 0.87^{ab}
F25.08	0.77 ± 0.31^{bc}	1.37 ± 0.64^{ab}	2.20 ± 0.89^{abc}
F25.09	0.23 ± 0.06^{a}	2.47 ± 0.25^{d}	2.97 ± 0.73^{bc}
F25.10	0.43 ± 0.38^{ab}	1.27 ± 0.35^{ab}	2.00 ± 0.26^{abc}

*Values with the same letter in the same column and in the same family are not significant different ($P < 0.05$).

Table 6: Heterosis values (%) by comparison of lesion necrotic size between parents and their progenies derived from F40 and F12 families.

Genotypes	**Day 2**	**Day 4**	**Day 6**
F40			
F40.01	51.79	65.5	65.25
F40.02	46.1	53.35	61.72
F40.03	28.14	24.19	28.92
F40.04	34.13	30.92	41.79
F40.05	34.13	-2.04	-5.29
F40.06	56.28	68.8	65.96
F40.07	46.1	72.88	68.78
F40.08	41.91	67.05	74.77
F40.09	58.08	67.93	67.72
F40.10	38.32	56.26	64.19
F40.11	35.92	55.39	68.25
F40.12	32.33	55.39	59.96
F40.13	100	41.6	53.61
F40.14	50.29	40.81	51.1
F12			
F12.01	53.89	46.68	57.58
F12.02	47.9	41.69	34.74
F12.03	51.79	67.93	74.77
F12.04	32.33	30.02	52.38
F12.05	47.9	44.6	39.5
F12.06	51.79	65.01	58.9
F12.07	59.88	57.14	62.96
F12.08	51.79	42.56	52.38
F12.09	23.95	51.31	58.9
F12.10	100	79.59	80.59
F12.11	56.28	52.47	62.96
F12.12	20.35	38.77	55.37
F12.13	50.29	49.5	20.1
F12.14	11.97	-7.87	15.34
F12.15	100	76.67	80.59

Table 7: Heterosis values (%) by comparison of lesion necrotic size between parents and their progenies derived from F20 and F25 families.

Genotypes	Day 2	Day 4	Day 6
F20			
F20.01	40.5	12.06	35.5
F20.02	76.34	43.23	46.85
F20.03	66.31	38.03	48.95
F20.04	49.82	32.1	48.11
F20.05	51.97	23.19	42.65
F20.06	87.81	9.83	22.9
F20.07	85.66	59.18	62.82
F20.08	64.16	44.34	52.31
F20.09	71.33	13.54	26.47
F20.10	85.66	56.59	63.66
F20.11	90.68	52.88	55.88
F20.12	90.68	18.37	27.94
F25			
F25.01	71.33	36.92	51.68
F25.02	69.18	58.07	64.92
F25.03	62.01	39.52	46.01
F25.04	64.16	49.17	51.68
F25.05	59.14	67.72	70.59
F25.06	51.97	43.23	50.21
F25.07	66.31	50.65	64.92
F25.08	44.8	49.17	53.78
F25.09	83.51	8.35	37.61
F25.10	69.18	52.88	57.98

Table 8: Evaluation of the heritability proper (h^2) of resistance to *P. megakarya* in the F40. F12. F20 and F25 cocoa families.

	Families	Crossing	h^2
1	F40	(♀)ICS_{40}× (♂) SCa_{12}	0.48
	F12	(♀) SCa_{12} × (♂) ICS_{40}	0.45
2	F20	(♀)ICS_{40} × (♂) SNK_{16}	0.65
	F25	(♀)SNK_{16} × (♂) ICS_{40}	0.47

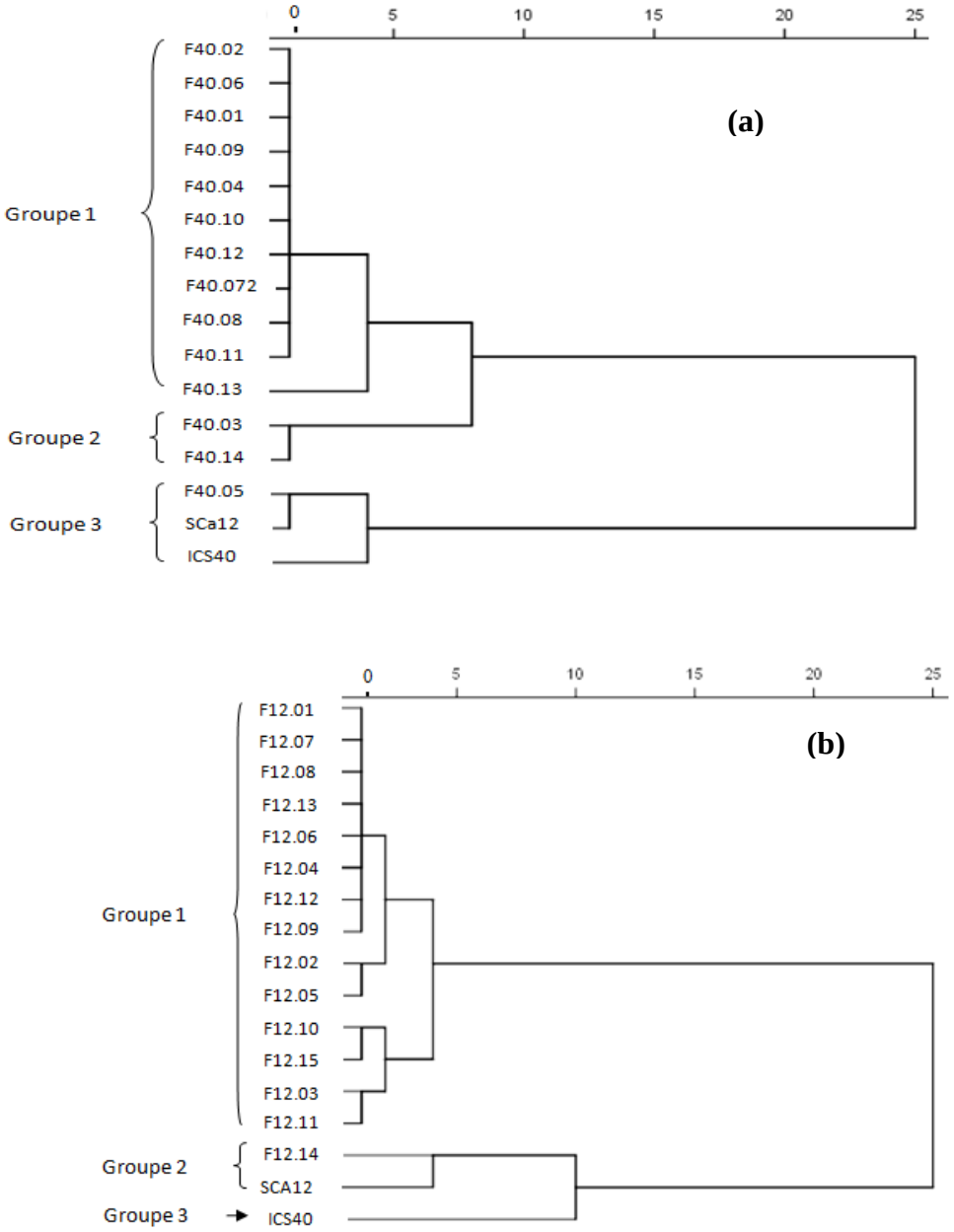

Figure 1: Direct hierarchical classification obtained with necrotic lesion size of leaves of ICS40, SCA12 clones and hybrids from F40 (a) and F12 (b) families of *T. cacao*.

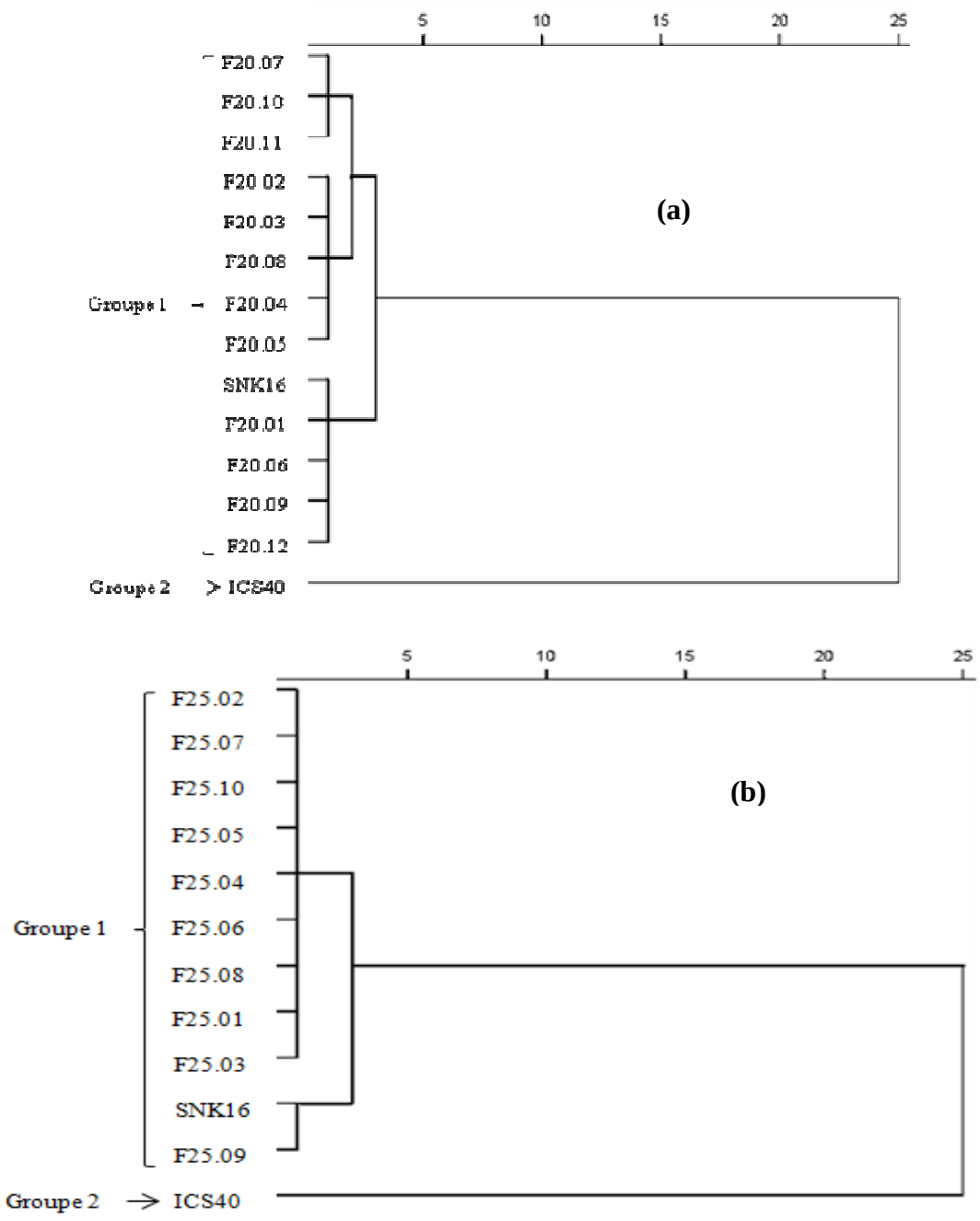

Figure 2: Direct hierarchical classification obtained with necrotic lesion size of leaves of ICS40, SNK16 clones and hybrids from F20 (a) and F25 (b) families of *T. cacao*.

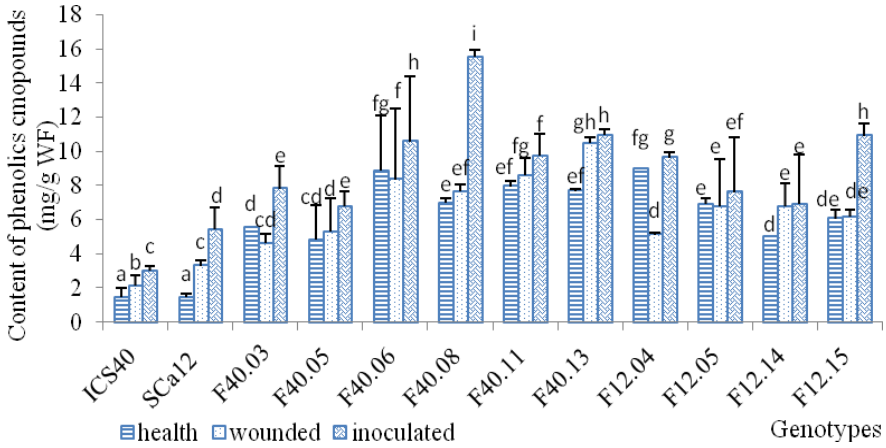

Figure 3: Phenolic content of clones (ICS40 and Sca12) and their progenies (F40 and F12 families) in different treatments.

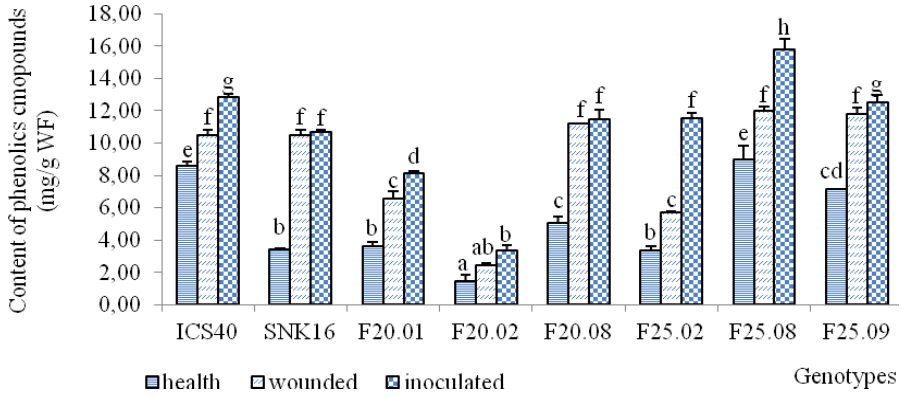

Figure 4: Phenolic content of clones (ICS40 and SNK16) and their progenies (F20 and F25 families) in different treatments.

DISCUSSION

Results of hand-pollinisation test were different according to the crosses used. In the first cross (F40: (♀) ICS40 × (♂) SCa12 and F12: (♀) SCa12 × (♂) ICS40), an average successful rate was obtained (46.15% and 36.06% respectively for F40 and F12). These results were similar to those found by Mossu (1990) in *T. cacao* and could be explained by the genetic compatibility of the two clones and the period of pollinisation.

This experiment allows us to analyse the heritability of resistance to *P. megakarya* by the evaluation of necrotic lesion size and phenolic compounds in healthy, wounded and inoculated leaves of ICS40, SNK16 and Sca12 clones and hybrids resulting from the reciprocal crossing. Results obtained show that wound inoculation of 1 or 2-month-old leaves of cocoa derived from the genotypes ICS40, SNK16 and Sca12, developed necrosis of the main vein. In general, the necrotic lesion is reduced in hybrid's genotypes compared to that of their parent. About 98% of the hybrid's genotypes manifested heterosis effect for the development of lesion size. This

suggests the existence of hybrid vigour with a genetic additive effect. The evolution of necrotic lesion size was more significant ($p<0.05$) in the sensitive ICS40 compared to Sca12. This symptom was also larger in the latter than in SNK16. These results are conformed to those documented by Nyasse et al. (2002) on cocoa leaf disks and by Omokolo et al. (2002) on cocoa pods. Similar results were also obtained by Djocgoue et al. (2007) when leaves of the ICS84 and ICS95 clones were inoculated with *P. megakaya*.

Some hybrids like F40.06, F40.07, F40.08, F40.09, F40.10, F10.10, F10.15, F20.7, F20.10, F25.02, F25.05 and F25.07 were characterized by small lesions. Excepted F40.05, F12.02, F12.05, F12.13, F20.09 and F20.12, the hybrids produced from the three clones were more tolerant to *P. megakarya* than the best parent SNK16. Also, no significant difference has been observed between the heritability of the lesion size for each of the two crosses (h^2= 0.48 and 0.45 for F40 and F12 respectively for the first cross, 0.65 and 0.47 for F20 and F25 in the second crossing). These results matched with those of Djocgoue et al. (2007) and are contradictory to those reported by Lockwood et al. (2007) where they showed a low heritability of the traits production and necrosis development in cocoa plants. In addition, in the experimental conditions of the present investigations, the parental and hybrid genotypes were planted in the same plot, and this had the effect of minimizing environment related effects, rendering the heritability estimations more trustworthy (Cilas, 1991). Regardless of the studied character, the absence of a significant difference between the heritability values from reciprocal crossing portrays the absence of maternal heritability. This observation suggests that the heritability of the development of necrosis is nuclear rather than cytoplasmic. This is in agreement with the investigations of Nyasse et al. (1995) after infection of disks of cocoa leaves with UPA134 x SNK64 and SNK64 x UPA164 by zoospores of *P. megakarya.* The works of Djocgoue et al. (2006, 2007) on leaves attached on the plant are also in agreement.

Clusters analyses differentiated each family in at least two groups. Individuals in the same groups displayed the same behaviour. Hybrids selected for the estimation of phenols were chosen randomly into the groups generated by direct hierarchical classification based on leaf necrotic lesion size.

In fact, one of the defence mechanism developed against pathogens is phenolic compounds. As far as responses to pathogen infections are concerned, one of the most significant responses is an increase in phenylpropanoid metabolism, which causes regional synthesis of phenolic compounds. The functional importance of this response is not yet completely understood. However, it is suggested that accumulation of phenolic polymers and lignin in the infection region to inhibit the invasion of the pathogen might be a result of increased synthesis of phenolics (Smirnoff, 2005).

In healthy plants, the tolerant clone SNK16 and Sca12 and 100% of the hybrid's genotypes have more phenolics than the sensitive clone ICS40. An increase of soluble phenolic content in stress conditions was also observed and this increase is more intense in infection condition. These findings are in agreement with results reported by Koc and Ustun (2012) who showed an increase of phenolic content in leaves of susceptible and resistant Pepper (*Capsicum annuum* L.) plants on day 6 following infection. Musseti et al. (2000) and Siranidou et al. (2002) also showed an increase of phenolic content in conditions of infection in potato's tissues and wheat's tissues respectively.

Some progenies (F40.06 and F40.08) showed an important increase in phenolics with small and restricted lesions in inoculated conditions. However, the F20.02 hybrid was particular; this hybrid has small lesions and low phenol content. This is in contrast to what is found in progenies from the (♀) SNK413 x (♂) SNK10 cross where hybrids, which produce small lesion and high concentration

of phenols were individuals where the female parent was the resistant clone (Boudjeko et al., 2006).

The objective of the present investigation was to search, in the framework of comparative hybrid tests of genotypes, for the most effective characters among those analysed and to identify the parental genotypes that show good combining potentials. The main results obtained showed that F40.08, F40.09, F40.13, F12.15, F25.02 and F25.08 showed an important increase of phenols with small and restricted lesions when inoculated with *P. megakarya*. These genotypes qualify themselves as the best ones since being the most tolerant. A mass multiplication of these genotypes and a bulk distribution to farmers is likely to boost cocoa farming in Cameroon. Furthermore, the parental genotypes (SNK16, SCa 12 and ICS40), show good aptitudes for the combination of the two studied characters, and their introduction into the biclonal planting field will be important.

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