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Comparative Assessment of the Resistance of Cocoa (*Theobroma cacao* L.) Progenies from SNK10 x SNK413; ICS84 x ICS95 to *Phytophthora megakarya* in Cameroon by Measuring Size of Necrotic Lesion along the Midrib

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Abstract: The susceptibility of *Theobroma cacao* L. to *Phytophthora megakarya* was assessed in four seedlings progenies (♀ ICS84 × ♂ ICS95, ♀ ISC95 × ♂ ICS84, ♀ SNK413 × ♂ SNK10, ♀ SNK10 × ♂ SNK413) by measuring daily, the size of the necrotic lesion along the midrib after inoculation. Using the statistical analysis of necrotic lesion along the midrib it was possible to show that progenies resulting from three clustured in 12 and 14 groups. The sizes of the lesion of parental clones are significantly different. The values of heterosis allow confirm the transmission of the vigor to the hybrid. For each progeny, more than 60% of individuals show this phenomenon. The ANOVA analysis of necrotic lesion shows a significant effect of day after inoculation and genotype to all the progenies at 0.001 probability level. Narrow sense heritability was average for SNK clones (local Trinitario) and high for ICS clones (introduced Trinatario), varying between 0.43 and 0.73. This suggested that ICS clones appeared to be the best promising parents than SNK. For every reciprocal crossing, no significant difference of narrow sense heritability was observed, this suggested the absence of a maternal effect when considering the size of necrosis.

Key words: *Theobroma cacao* (cocoa), *Phytophthora megakarya*, progenies, resistance, heritability

INTRODUCTION

New approaches of plant-breeding research programs in Cameroon have well defined objectives, which should be economically, biologically reasonable and environmental sound. Economic criteria depend on the production of varieties, that farmers and users actually want. Biological objectives are determined by the yield and quality (Simmonds and Smartt, 1999). Regarding the impact of black pod disease pressure on cocoa yields, resistance to this disease is the major character taken into account by plant breeders. If resistance alone was all that mattered, resistance breeding would often be easy; the difficulties lie in combining sufficient resistance with other characters so as to produce a satisfactory variety.

Cocoa (*Theobroma cacao* L.) provides a substantial income for smallholders in the Tropics. 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, 1985). In Cameroon, the disease is of a particular importance due to the existence of a single species of *Phytophthora megakarya*, considered to be the most aggressive in the field (Nyasse *et al*., 1995; Omokolo *et al*., 2003). Chemical control of such a disease is expensive and both commercially and environmentally needless. It can nevertheless be used temporary, but disease resistance is often a major, if not a dominant feature of many plant-breeding programs. Other methods available for controlling cocoa black pod rot are the use of resistant cultivars and appropriate cultural practices such as phytosanitary pod removal which is a potentially efficient control method (Ndoumbe-Nkeng *et al*., 2004).

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In Cameroon most cultivated cocoa trees derived from old varieties early introduced by the German colons, and the F2 generation seeds obtained from new hybrid cultivars. Hybrid selection is based on heterosis observed in crossing genetically distinct genotypes. Local and introduced clones available in the gene banks were generally used as hybrid progenitors. Yield capacity, sensibility and vigor of such hybrids have been used on a large scale. However, these hybrids have generally provided no satisfaction in obtaining good disease resistance and the yield often declined after 5 to 10 years. Moreover, the mixed hybrids varieties have also shown large phenotypic variation for all traits, often not desired by farmers (Ndoumbe-Nkeng *et al.*, 2001). Cocoa breeders continues to face the problem of the high heterogeneity between individuals derived from one cross and heterogenous transmission of genetic traits to the progeny. In this study early screening tests have been applied on nursery material in order to evaluated 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: Four cocoa clones available in gene banks of the Institute of Agricultural Research for Development (IRAD) at Nkoemvone Research Station (southern Cameroon) are used to created four progenies: two local Trinitario (SNK10, SNK413) and two Trinitario introduced from Trinidad (ICS95, ICS84). Crossing were realized in Nkoemvone Research Station of IRAD on March and April 2000 using hand-pollinisation techniques (Cilas, 1991). The four progenies obtained was:

F30: ♀ ICS84 × ♂ ICS95
F25: ♀ ISC95 ×♂ ICS84
F16: ♀ SNK413 ×♂ SNK10
F3: ♀ SNK10 ×♂ SNK413

Production of seedlings and grafts: Seeds from pods harvested in experimental field are sown in the nursery at Teacher's Higher Training College at University of Yaoundé 1 (Cameroon) and 389 hybrids plants were obtained. Parental plantlets were obtained through top-grafting by using budwood from the four clones listed above.

Leaf inoculation and analysis: The leaf test is an artificial inoculation method that can be used to asses the resistance of genotypes. Briefly, whole leaves from one or two months old plants were washed thoroughly with tap water and sterilized with ethanol 70. 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 *Phytophthora megakarya* obtained from a 7 days old culture and incubated at 25-26°C in the total dark. Control leaves were inoculated with sterile agar disc in the same conditions. The isolate uses of *Phytophthora megakarya* collected from a naturally infested pod from the Nkolbisson station. Necrotic lesions appear two days after inoculation and the size of these lesions was measured daily until day 6.

In order to emphasize on the effects of sex of parents on transmission of resistance, two genetic parameters were estimated: the heterosis (Zahour, 1992) and the inheritance (Cilas, 1991). The comparative analysis was done between parents and their progenies using length of necrose.

Statistical analyses: Hierarchical classification of parents and their progenies was obtained by using principal component analysis (PCA). ANOVA and Newman and Keuls test permitted to compare the susceptibility level of better progenies resulting from different crosses and to assess hybrid vigor (Begun and Gabriel, 1981). ANOVA and PCA were performed using SAS-system (Anonymous, 1997).

RESULTS

The disease severity was obtained by measuring daily basis the size of the lesion along the midrib two to six days after inoculation. PCA enabled the classification of individual of different hybrid families into 14, 13, 6 and 12 groups respectively for F30, F25, F16 and F3 progenies (Fig. 1-4). Results of inoculation test showed significant differences among parental clones. Newman and Keuls test displayed individuals that were less susceptible than their best parents. They are: F3012, F30153, F30146, F30171, F30124, F30139, F30100 and F30198 for F30 progenies (Table 1); F2557, F2556, F2552 for F25 progenies (Table 2); F1624 for F16 progenies (Table 3) and F332, F326 and F327 for F3 progenies (Table 4).

The heterosis value obtained by a comparison of the evolution of necrotic lesion size on midrib between the parent average and progenies confirm the vigor hybrid of those genotypes: F30124 (67%), F30100 (63%), F30198 (66%), F30146 (61%), F2552 (80%), F2557 (59%), F2586

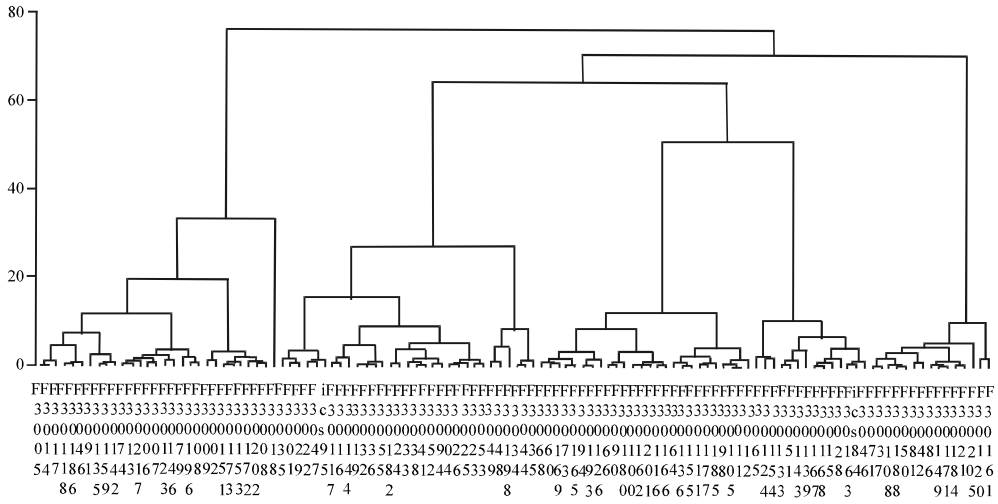

Fig. 1: Dendrogram showing the diversity of F30 progenies based on PCA of necrotic size of the lesion along the midrib from day 2 to day 6

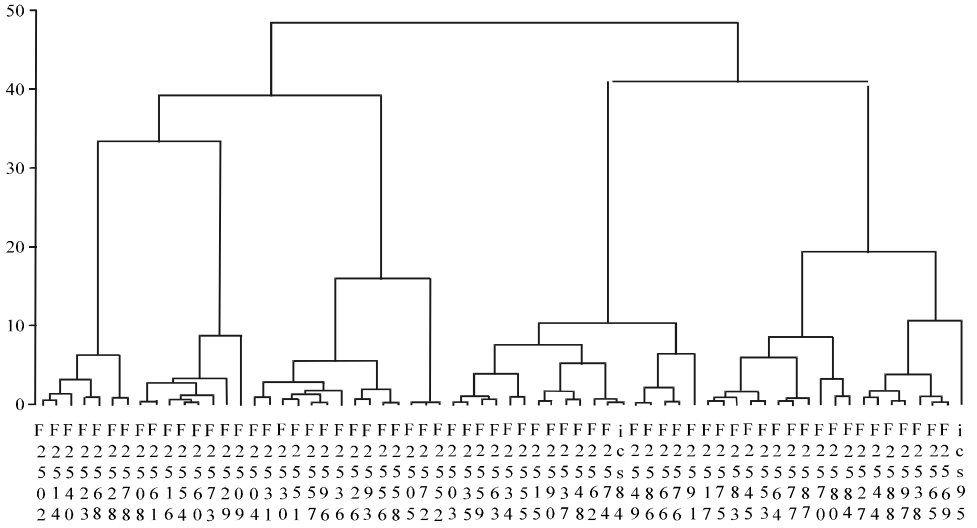

Fig. 2: Dendrogram showing the diversity of F25 progenies based on PCA of necrotic size of the lesion along the midrib from day 2 to day 6

(51%), F1624 (90%), F1621 (70%), F1606 (54%), F326 (60%), F332 (59%) and F327 (54%). Three days after inoculation 71% and 100% of hybrid respectively for F30 and F25 family revealed the vigor effect. Whereas for F16 and F3 family, five days after inoculation, 67 % of hybrid show the vigor effect. The ANOVA test on necrotic lesion shows a significant effect of day and genotype to all the progenies at the 0.001 probability level. A significant interaction between day and genotype was also observed at the same probability. Apart from midrib tissue, evolution of necrotic lesion continued on the limb. The results obtained were similar to those of midrib. However the lesion size observed was 10 times less than that of the midrib (data not shown).

For each progeny, narrow sense heritability (h^2) was estimated using the slope of the regression strain between average size of necrosis of the parents and progenies (Fig. 5). The values of narrow sense heritability are:

Progenies	Crosses	h^2 values
F30:	♀ ICS84 × ♂ ICS95	$h^2 = 0.70$
F25:	♀ ISC95 × ♂ ICS84	$h^2 = 0.73$
F16:	♀ SNK413 × ♂ SNK10	$h^2 = 0.43$
F3 :	♀ SNK10 × ♂ SNK413	$h^2 = 0.54$

Table 1: Average lesion size (cm) evolution on midrib of parents and F30 progenies

	Times (Days)				
Genotypes	J_2	J_3	J_4	J_5	J_6
F_{3018}	2.47±0.25^{a}	5.17±0.20^{a}	6.97±0.37^{a}	8.02±1.08^{a}	8.75±1.80^{ab}
F_{3022}	2.25±0.29^{ab}	4.20±0.49^{ab}	4.85±1.10^{ab}	5.32±1.36^{abcd}	5.77±1.18^{bcde}
ICS95	2.20±0.34^{ab}	4.25±0.48^{ab}	5.44±0.77^{abc}	6.63±0.91^{abc}	7.47±0.96^{abc}
F_{3019}	1.70±0.21^{bc}	3.85±0.36^{bc}	5.17±0.31^{abc}	5.47±0.15^{abcd}	5.82±0.18^{bcde}
F_{3038}	1.60±0.29^{c}	3.75±0.29^{bc}	6.22±0.59^{ab}	7.75±1.1^{a}	8.90±1.60^{ab}
ICS84	1.41±025^{cd}	3.20±0.46^{bcd}	5.27±0.85^{abc}	7.39±0.95^{a}	9.19±0.85^{ab}
F_{3012}	1.15±0.05^{cde}	1.85±0.24^{efg}	2.37±0.58^{d}	2.90±0.73^{d}	3.01±0.70^{e}
F_{30153}	0.95±0.05^{def}	1.97±0.31^{defg}	3.30±0.25^{cd}	5.10±0.40^{abcd}	6.67±0.00^{abcd}
F_{30146}	0.86±0.33^{def}	1.43±0.76^{fg}	3.03±0.50^{cd}	4.06±0.75^{cd}	4.73±0.38^{cd}
F_{30171}	0.86±0.25^{def}	2.40±0.25^{defg}	3.13±0.10^{cd}	4.30±0.21^{cd}	5.06±0.49^{cde}
F_{30124}	0.75±0.20^{def}	1.22±0.17^{g}	2.30±0.32^{d}	3.080±0.23^{cd}	5.82±0.62^{bcde}
F_{30139}	0.75±0.05^{def}	2.75±0.25^{cdef}	4.90±0.20^{abc}	6.80±0.50^{abc}	9.60±0.60^{a}
F_{30100}	0.70±0.21^{def}	1.36±0.45^{g}	2.40±0.49^{d}	3.00±0.85^{d}	3.73±0.55^{de}
F_{3091}	0.67±0.02^{def}	1.55±0.49^{fg}	3.17±1.05^{cd}	4.72±0.5^{bcd}	6.32±0.52^{abcde}
F_{30198}	0.62±0.19^{ef}	1.25±0.37^{g}	1.97±0.63^{d}	5.37±0.57^{abcd}	5.87±0.70^{bcde}
F_{30161}	0.30±0.00^{f}	3.00±0.18^{bcde}	3.90±0.18^{bcd}	5.30±0.99^{abcd}	6.45±0.74^{abcde}

Means followed by the same letter(s) in the vertical column are significantly different at $p<0.05$

Table 2: Average lesion size (cm) evolution on midrib of parents and F25 progenies

	Times (Days)				
Genotypes	J_2	J_3	J_4	J_5	J_6
ICS95	2.20±0.34^{a}	4.25±0.48^{a}	5.44±0.77^{abc}	6.63±0.91^{abc}	7.47±0.96^{abcd}
F_{2591}	1.97±0.28^{ab}	3.30±0.49^{ab}	6.97±1.08^{a}	7.47±1.04^{ab}	8.20±0.68^{ab}
F_{2588}	1.92±0.14^{abc}	3.55±0.26^{ab}	6.22±0.10^{ab}	6.80±0.34^{abc}	7.12±0.34^{abcd}
F_{2537}	1.56±0.12^{bcd}	3.60±0.29^{ab}	5.23±0.12^{abcd}	6.66±0.62^{abc}	8.63±0.34^{ab}
F_{2580}	1.47±0.22^{bcde}	2.42±0.26^{bc}	6.67±0.58^{a}	7.85±0.90^{a}	8.42±1.14^{ab}
ICS84	1.41±0.25^{cde}	3.20±0.46^{ab}	5.27±0.85^{abcd}	7.39±0.95^{ab}	9.19±0.85^{a}
F_{2577}	1.27±0.04^{de}	3.27±0.57^{ab}	5.800.64^{abc}	7.27±0.21^{abc}	8.00±0.61^{abc}
F_{2568}	1.27±0.19^{de}	3.27±0.28^{ab}	4.97±0.52^{abcd}	7.12±0.43^{abcd}	7.70±0.80^{abcd}
F_{2557}	1.25±0.26^{de}	1.50±0.22^{e}	2.57±1.02^{ef}	2.72±1.06^{ef}	2.82±1.06^{e}
F_{2561}	1.25±0.23^{de}	3.57±0.12^{ab}	4.05±0.08^{cde}	5.02±0.50^{cd}	5.420.46^{cd}
F_{2556}	1.15±0.26^{de}	2.47±0.60^{bc}	2.82±0.75^{ef}	2.90±0.71^{ef}	3.00±0.70^{e}
F_{2563}	1.07±0.08^{de}	2.97±0.28^{b}	4.450.99^{bcde}	5.25±0.70^{bcd}	6.40±1.14^{abcd}
F_{2586}	0.85±0.09^{ef}	1.82±0.59^{ab}	3.67±0.74^{cde}	4.55±0.94^{de}	6.17±1.10^{bcd}
F_{2552}	0.52±0.12^{fg}	0.72±0.24^{d}	1.30±0.52^{f}	1.65±0.52^{f}	3.22±0.45^{e}
F_{2509}	0.35±0.05^{g}	2.85±0.65^{b}	3.25±0.75^{de}	3.55±0.95^{def}	5.25±1.25^{d}

Means followed by the same letter(s) in the vertical column are significantly different at $p<0.05$

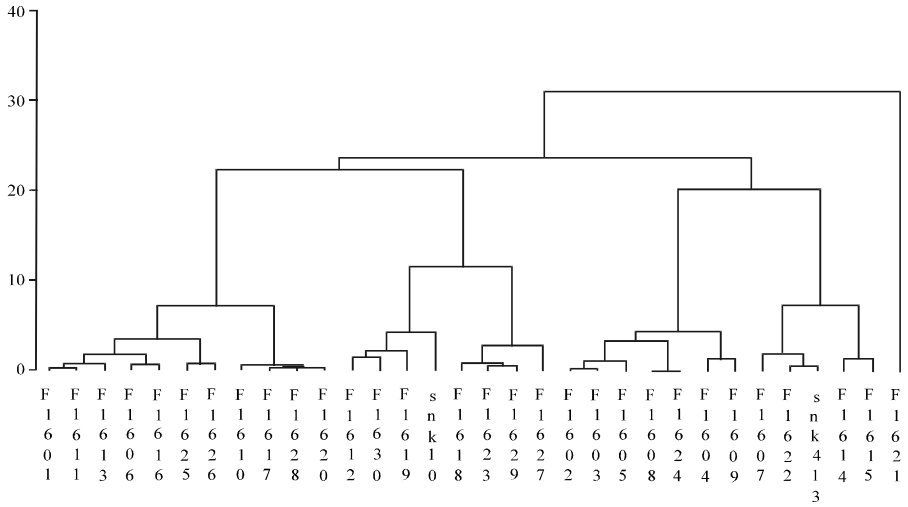

Fig. 3: Dendrogram showing the diversity of F16 progenies based on PCA of necrotic size of the lesion along the midrib from day 2 to day 6

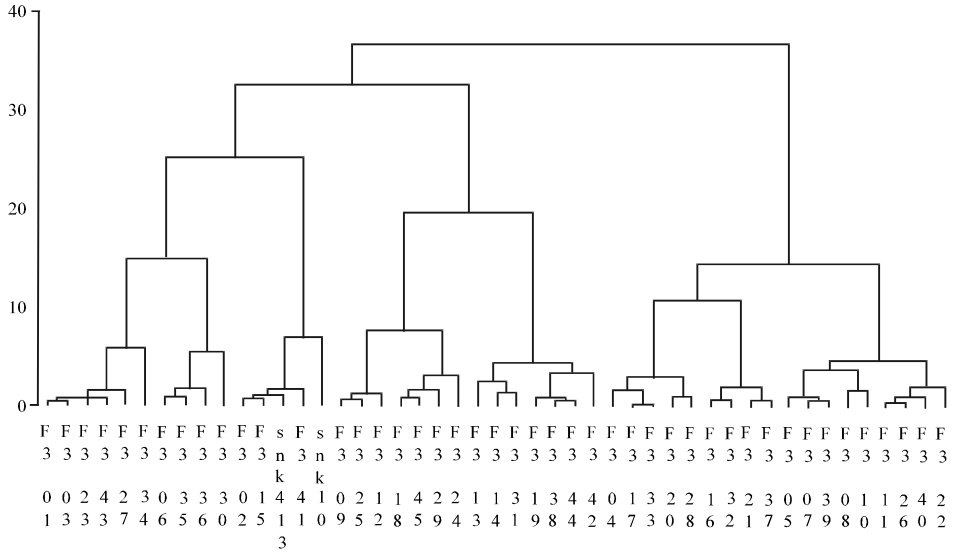

Fig. 4: Dendrogram showing the diversity of F3 progenies based on PCA of necrotic size of the lesion along the midrib from day 2 to day 6

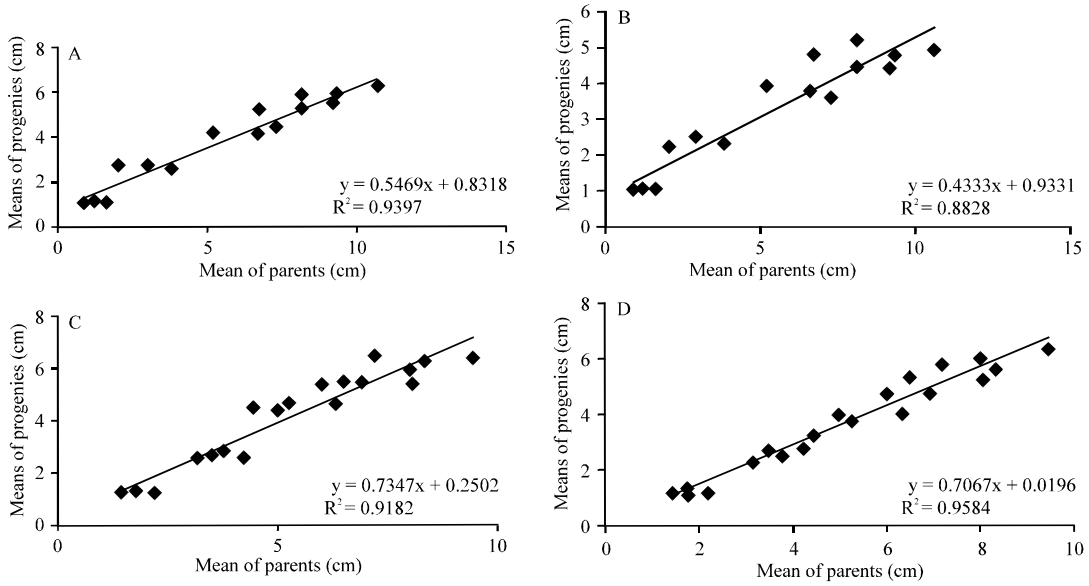

Fig. 5: Evaluation of narrow sense heritabilities (h^2) using the regression slope between parental genotypes and their progenies: F3 progenies (A); F16 progenies (B); F25 progenies (C) and F30 progenies (D)

Table 3: Average lesion size (cm) evolution on midrib of parents and F16 progenies

	Times (Days)				
Genotypes	J_2	J_3	J_4	J_5	J_6
SNK10	1.78±0.48^{a}	4.31±0.92^{a}	8.37±1.00^{a}	9.88±1.30^{a}	11.39±1.28^{a}
F1614	1.50±0.00^{ab}	2.60±0.00^{bc}	3.57±0.33^{cd}	4.87±0.24^{cd}	5.06±0.05^{c}
F1620	1.47±0.25^{ab}	3.47±0.50^{ab}	5.70±0.22^{b}	6.33±0.24^{c}	6.60±0.33^{b}
F1606	1.27±0.21^{abc}	3.03±0.05^{abc}	3.37±0.12^{cd}	4.10±0.64^{d}	4.37±0.68^{c}
F1619	0.90±0.22^{bcd}	2.43±0.61^{bc}	6.47±0.45^{b}	8.30±0.50^{b}	10.20±0.57^{a}
F1621	0.73±0.19^{cd}	2.27±0.52^{bc}	2.60±0.62^{d}	2.73±0.61^{e}	2.77±0.57^{d}
SNK413	0.71±0.19^{cd}	1.54±0.34^{cd}	4.37±0.99^{c}	6.21±0.62^{c}	7.39±1.04^{b}
F1624	0.40±0.08^{d}	0.53±0.12^{d}	1.03±0.05^{e}	1.03±0.05^{f}	1.03±0.05^{e}

Means followed by the same letter(s) in the vertical column are significantly different at $p<0.05$

Table 4: Average lesion size (cm) evolution on midrib of parents and F3 progenies

	Times (Days)				
Genotypes	J_2	J_3	J_4	J_5	J_6
F324	2.00±0.00^{a}	4.00±0.16^{ab}	6.00±0.41^{bc}	6.30±0.43^{bc}	6.63±0.45^{bc}
SNK10	1.78±0.48^{ab}	4.31±0.92^{a}	8.37±1.00^{a}	9.88±1.30^{a}	11.39±1.28^{a}
F345	1.77±0.21^{ab}	3.67±0.62^{ab}	6.23±0.21^{b}	7.50±0.08^{b}	8.23±0.21^{b}
F330	1.37±0.12^{bc}	4.60± 0.08^{a}	5.43±0.31^{bcd}	6.06±0.33^{bc}	6.63±0.45^{bc}
F306	1.25±0.24^{bc}	4.55±0.05^{a}	6.85±0.35^{b}	7.75±0.25^{b}	8.60±0.60^{b}
F339	1.13±0.12^{cd}	2.43±0.76^{c}	3.80±0.86^{def}	4.17±1.09^{de}	4.33±1.11^{d}
F317	1.10±0.05^{cd}	2.30±0.45^{c}	3.80±0.60^{def}	5.50±0.50^{cd}	6.50±0.90^{bc}
F326	1.10±0.08^{cd}	1.63±0.26^{c}	2.63±0.26^{f}	3.13±0.12^{e}	3.77±0.21^{d}
F314	0.80±0.14^{cd}	2.90±0.14^{bc}	4.60±0.65^{cde}	7.00±0.41^{bc}	7.50±0.78^{bc}
F342	0.80±0.12^{cd}	1.60±0.22^{c}	2.67±0.62^{f}	5.23±0.21^{cd}	5.60±0.29^{cd}
SNK413	0.71±0.19^{cd}	1.54±0.34^{c}	4.37±0.99^{cdef}	6.21±0.62^{bc}	7.39±1.04^{bc}
F334	0.70±0.08^{cd}	2.03±0.03^{c}	3.40±0.08^{ef}	5.30±0.08^{cd}	7.17±0.12^{bc}
F327	0.53±0.05^{d}	2.13±0.12^{c}	2.47±0.12^{f}	3.43±0.17^{e}	4.30±0.22^{d}
F332	0.50±0.08^{d}	2.13±0.12^{c}	3.30±0.41^{ef}	3.50±0.41^{ef}	3.83±0.29^{d}

Means followed by the same letter(s) in the vertical column are significantly different at $p<0.05$

Moreover there was a significant correlation for necrotic size between parents and progenies during the crossing.

DISCUSSION

The study has enabled the selection of progenies from SNK10 x SNK413; ICS84 x ICS95 for their resistance to *Phytophthora megakarya*. Because of the performance recorded in their off-springs (heritability), the most resistant progenity could be cropped to assess other traits useful for breeding, such as yield. The heterosis gained suggests the good combining ability of the progenitors used. A similar study was carried out in Cameroon by observing the incidence of natural infection in the field (Nyassé *et al*., 1995). The general combining ability effect was substantial for the pod rot rate (Cilas *et al*., 2004), meaning that the characters are primarily transmitted additively (Tan and Tan, 1990). Since the heritability values from field incidence of the disease increase with the number of years of observations (Cilas *et al*., 2004), we used a leaf test which have the advantage of giving significant values of heritability within a short selection cycle. Although correlation with leaf test between organs taken from field plants and those from nursery plants is not very high due to environmental effects (Tahi *et al*., 2000), it was shown that correlation between the leaf test and pod rot rate in the field was related to the genetic constitution of plant and not to environmental conditions (Nyasse *et al*., 2002). Then, the field resistance of cacao genotypes to black pod disease can be predicted using early screening test on nursery plant material.

Different crosses revealed the absence of the maternal effect. This observation implies that progeny involved in the partial resistance of cocoa to *Phythophthora* sp are nuclear and not cytoplasmic. Moreover, in the progenies some individuals plants are more effective than the best parents. Heterosis value confirms these observations. Genotypes used in this crossing program were of the Trinitario group, which are highly heterozygous (Laurent *et al*., 1995). In order to increase the level of homogeneity of the progenies distributed to cocoa farmers, the use of several cycles for the selection of progenitors, to increase their homozygosity, is not being considered a promising method. Indeed, this is an excessively time consuming method, since four or five years are required per cycle (Tan and Tan, 1990). In practice, it is found that to inbreed any normally out breed organism it should have fairly stromatic effects, especially upon finless characters. There is always a decline in general vigor, size and fertility. The decline is such that, with close inbreeding, some or many inbreed become non viable. They simply die due to an inbreeding depression (Simmonds and Smartt, 1999). What ever the interpretation, the fact that heterozygotes are (not always) fitter than homozygotes is well established. They are also often more stable to face environmental variation. Heterozygosity therefore often promotes stability performance. Stability however, is a feature not only of heterozygosity, but also of general genetic constitution (Simmonds and Smartt, 1999).

In Cameroon, only about 21% of *T. cacao* cultivated today are of selected varieties. The other 79% of trees are traditional populations. The selected varieties were obtained from SNK, having characteristics similar to those of the Criollo group (high susceptibility to *Phytophthora* disease) (Laurent *et al*., 1994). The breeder needs to reconsider the parents' clones used in the breeding scheme to improve the yield in the cocoa field. In this study, ICS genotypes appear to be better parents than SNK. ICS are introduced Trinitario, characterized by vigor and less susceptible or resistant to disease

(Laurent *et al*., 1994). Thus, the relationship between resistance of parental clones and their off-springs allows the identification of ICS95 as a suitable parent for the accumulation of resistance genes in cocoa populations (Evans *et al*., 1998). Also, a clone like ICS84 can be used as a promising parent because of its good predicting value to transmit resistance to *Phythophthora megakarya*. This result agrees with findings of Tan and Tan (1990) in cocoa vis à vis of *P. palmivora*.

The purpose of this study was to select progenies resistant to *P. megakarya*. Our results showed that for F30 (♀ ICS84 × ♂ ICS95), F25 (♀ ISC95 × ♂ ICS84), F3 (♀ SNK10 × ♂ SNK413) and F16 (♀ SNK413 × ♂ SNK10) progenies, 9 (F3012, F30153, F30146, F30171, F30124, F30139, F30100 and F30198), 3 (F2557, F2556, F2552), 3 (F332, F326 and F327) and 1 (F1624) individuals were less susceptible to *P. megakarya* than their better parents. However, to confirm these findings, further work could be carried out in the field. If the potential of interesting individuals are confirmed in the field following the results that will be analysed in the near future, then large scale distribution of these progenies to cocoa farmers will be beneficial.

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