Original Research Article

Assessing the tolerance of cocoa (*Theobroma cacao* L.) progenies to the black pod disease caused by *Phytophthora megakarya* Bras. and Griff.

ABSTRACT

The cocoa (*Theobroma cacao* L.) is a cash crop of great economic importance to some cocoa-growing countries. However, diseases and pests, including black pod disease caused by *Phytophthora megakarya*, can cause yield losses of up to 100% if no phytosanitary treatment is applied. Thus, the aim of this work is to evaluate the tolerance of five cocoa hybrids developed by IRAD (PA107*SNK614, IMC67*SNK109, SCA12*SNK16, IMC67*SNK64 and T79/501*SNK64) against black pod disease in relation to the parent clones. Mature pods (approximately 5 months old) were harvested and used to assess black pod tolerance using the Iwaro Detached Pod Test (DPT-SM). Means were separated using the general linear model (GLM) and ranked using the Student Newman-Keuls multiple comparison test (SNK). ANOVA showed a significant difference at P=0.01 on tolerance between hybrids and P=0.001 on tolerance between hybrids and clonal groups. 40% of the hybrids tested were more tolerant than all the clonal groups. The IMC clonal group was the most tolerant of the six clonal groups tested and ranked third overall between hybrids and clonal groups tested. Although the tolerance score varied between hybrids of clones PA107*SNK614 was the most tolerant (0.79) and T75/501*SNK 64 being least tolerant (2.00), all of these hybrids of clones possesses real potential to the tolerant to the black pod disease.

Keywords: *Theobroma cacao*, *Phytophthora megakarya,* Tolerance, Hybrids of clones, Clones.

1. INTRODUCTION

The cocoa tree (*Theobroma cacao* L.) belongs to the Malvaceae family [25]. This diploid plant (2n=20) is native to tropical America from where it migrated to other countries all over the world [30]. It is cultivated for its beans which constitute the raw material of several products (chocolate, cocoa butter, etc.) in the cosmetics and in pharmacology industry [8].In 2021, West Africa produced almost 2/3 of the global production with Côte d'Ivoire producing 2.2 million metric tons and ranked as highest producer, Ghana as second highest producer(1.1million metric tons) while Cameroon came 5th in the ranking with 295,163 metric tons [6]. According to the ONCC statistics 2021, Cameroon produced 295,163 metric tons of cocoa beans accounting for 6% of the world's production [7]. The ICCO statistics for 2022 estimates global production to be 5,240 metric tons [6].However, cocoa's productivity is limited by several abiotic and biotic constraints among which is the black pod disease that is said to be the most destructive[10]. The Black pod disease caused by the *Phytophthora* spp.is said to be the main and most destructive disease in cocoa plantations[20]. Four species of *Phytophthora* have been identified as responsible for this disease: *P. palmivora, P. megakarya, P.*

*capsici*and *P. citrophthora*[18]. In Cameroon studies by [35] revealed the presence of *P. megakarya* as the predominant specie. It can cause up to 50-80% reduction in production [36]. Field losses can reach 100% if no protection measures are taken. The pathogen *P. megakarya* can survive out of the host for up to 10 months, once the conditions (environmental) are favorable the pathogen migrates from the soil to different parts of the plant where they infect and plant and cause the disease[28] .However, different control measures exist for the control of the disease, they include the chemical control, biological control, the integrated pest management (IPM) and the genetic control.

Chemical (systemic and contact) fungicides are the most widely used method for the control of the black pod disease but they cause considerable damage to the environment and on the health of the farmers and the consumers of cocoa products[1]. The biological control is safer since it makes use of microorganisms and plant extracts, but it has several limitations[14].

The agronomic control on the other hand is environmentally friendly and has no negative impact on the human health, but it is not as effective as the chemical control against the black pod disease[2]. The genetic control measures make use of plant improvement techniques to control the disease[17]. However, integrated pest management (IPM) which makes use of a combination of several management practices have proven to be efficient if properly manage. This is limited as it cannot be effectively practiced in large plantations, it requires time and a mastery of some skills.In Cameroon the first group of hybrids were developed between the 1960s and the 1980s and the focus was mostly on high yield traits. In the early 2000, news hybrids were developed by IRAD. These hybrids were developed with parental clones located at the IRAD germplasm collections and the focus was on improving yield and the tolerance to the black pod disease caused by the *P.megakrya* which till date pose a serious challenge to the production of cocoa beans. Trials were established on-station at IRAD. On-farm trials were equally established on farmer's field and are ongoing. It is hypothesized that hybrids will show more tolerance to the black pod disease (caused by the *P. megakarya*) than the parent clones. Theobjective was to compare the tolerance of clones and hybrids of clones to the black pod disease caused by *Phytophthora* spp.

2. MATERIAL AND METHODS

2.1 Material

2.1.1 Presentation of the study site

The plant material (clones) used for the study were obtained from the IRAD gene bank at the Nkoemnvone research stations in the South Region. The hybrids of clones on the other hand are planted in on-farm trials on farmers' fields situated at Kedia in the Bokito sub-division (Mbam et Inoubou Division) with coordinates 4°30'00" North, 10°06'00" East, mean annual temperature of 25.5 °C and rainfall of 1500-1700 mm. The trials were established by the IRAD Cocoa Research team in 2005 as a part of the Regional Variety Trial (on-farm) (fig. 1). On the other hand, the artificial inoculation tests were carried out in a confined environment at the Plant Pathology Laboratory at the Agricultural Research Institute for Development (IRAD) in Nkolbisson.

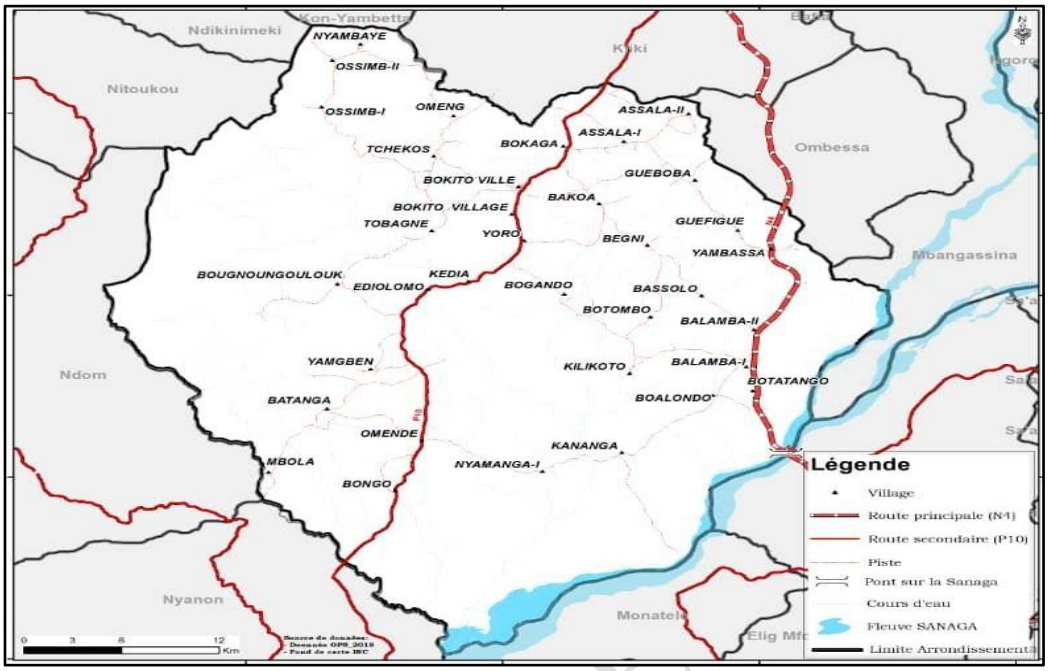

Fig.1.Localization of the study site (Atlas Forestier 2020).

2.1.2 Plant Material

The plant material used for the study are composed of hybrids of clones and parental clones. A total of 5 hybrids of clones originating from crosses with clones (obtained from the germplasm at IRAD Nkoemnvone Research Station). A total of 3 individuals were selected from each progeny from which mature pods (4-5 months) was collected to carry out the test for the resistance to the black pod disease. Six clonal groups made up of 22 clones belonging to various genetic sub-groups (Trinitario and Forestero), were selected from the gene bank to be used as control clones. Of the 8 parents used, 4 are used as female and 4 as male parents. However, IMC 67 is used twice as a female parent and SNK 64 used twice as male.

- Three Upper Amazonian Forastero (UAF):IMC 67, PA 150, SCA 12.
- Three Trinitario (Tr): SNK13, SNK64, SNK 109, SNK 614.

A total of 3 individuals were randomly selected within each of the 5 hybrids of clones used in the study. Cocoa pods were equally harvested from the cocoa germplasm at the Nkoemvone research station. For each time repetition, two cocoa pods were collected for the test of the level of tolerance of the black pod disease per progeny.

2.1.3 Fungi Material

The fungi material used is a moderately aggressive isolate *P.megakarya*, conserved at the Central PlantPathology Laboratory of IRAD, Nkolbisson. These isolates were obtained from*Phytophthora* spores isolated and characterized at Ebolowa before being transferred to the Laboratory. These and many others are currently under culture to preserve its viability.

Three isolates (LBEBW 1-289, LBEBW 1-196 and LBEBW 1-235) to be used in the experiment were grown on the PDA. After the preparation of the culture medium, each of the isolates was dropped on the Petri dish containing the media and labeled. The set up was kept in a dark carbonate for 10 days after which the growth of the 3 isolates on the PDA was evaluated. The isolate LBEBW 1-289 was selected as the averagely virulent isolate and used for the test[38].

The zoospores from LBEBW 1-289 were obtained from a 7 days old culture of the isolate. The spores were incubated for 5 days in a dark chamber at 25^{o}C. The culture is later transferred and incubated for 5 more days in the light. During this time sporangia will form and mature. The plate is flooded with cold, sterile distilled water (4^{o}C) and incubated for 20-45 min at 4^{o}C and then moved to a dark chamber at 25^{o}C for 20–30 min during which time the zoospores are released[39].

At the Laboratory equipment such as the light microscope, a hand sprayer, the micro pipette, hectospectometer meter (Malassez Cell) and plastic bowls were used during the test.

2.2 METHODS

2.2.1 Preparation of the pod

The pods aged 5 to 6 monthsold that were fully mature but not ripe were harvested between 8 and 11 am. They were wrapped in tissue paper, put in a plastic for proper protection and transportation to the Laboratory. Once in the laboratory the pods were washed with tap water.

2.2.2 Experimental setup

The material used are plastic bowls, spongy material, paper tags (for labeling) tissue paper and 100ml measuring cylinder. The bottom of the plastic trays used for the experiment were lined with a spongy material and covered with blotted paper. Using a measuring cylinder, 100ml of distilled water was used to moisten the blotted paper in each tray. The pods are washed with tap water, labeled and placed in the moist plastic bowl.

2.2.3 Preparation of culture medium and inoculation

The culture medium used was the Potatoes dextrose agar PDA, 39g of PDA was dissolved in 1000 ml of distilled H_2O. The 1000 ml the solution was homogenized using a magnetic stirrer, then sterilized in an autoclave for 20 min at a temperature of 125°C[49].The medium was left to cool down before taken to the wood for the culturing of the *P. megakarya.* The *P. megakarya* was cultures for 7 days.

2.2.4 Isolation and calibration

Spores were collected from the periphery of the growing mass of fruiting body in the Petri dish, as this is where there actively growing cells are located. Sterile distilled water(10ml) was added to the beaker contain the spores. Spore liberation was favored by subjecting the mixture to a temperature of 4°C in a refrigerator for 5 minutes, then in ambient temperature in a dark environment for 25 min.

The spore concentration was adjusted in the solution before being used to infect the pods. Calibration was done by spore counting using of a hectospectometer (Malassez cell). This suspension was calibrated at approximately 3 x 10^5 spores/ml following the protocol developed by[36] .

2.2.5 Inoculation of the pods

The upper surface of the pod was sprayed with the suspension of 3 x 10^5 spores/ml of zoospores of *P.megakarya*using a chromist atomizer at a 30cm distance. After inoculation, the trays were covered with

glass and kept to incubate in a culture room at temperatures between 24 and 26 °C away from light for 5 days. The symptoms were read using the rating scaleproposed by[23].

2.2.6 Statistical Data Analysis

Data from the detached pods were entered on an Excel spreadsheet (2016) and analyzed by SAS (2002) software version 8 for the comparison of variation among hybrids of clones, variation within hybrids of clones and variation between hybrids of clones and clones. The GLM procedure using the Student Newman-Keuls test [12] at the 5% probability threshold was used to first compare the mean within the hybrids of clones, between the hybrids of clones and that of the clones. The mean score of the tolerance *to P.megakarya* was then ranked among the individuals of the progeny, between the hybrids of clonesand between the hybrids of clones and the clones.

3. RESULTS AND DISCUSSION

3.1Results

3.1.1 Necrosis development and pathogen isolation

The level of tolerance to the black pod disease of the five hybrids of clonescreated by the IRAD cocoa breeding program and planted in on-farm trials was evaluated and a comparison made between the hybrids of clones and some clonal groups.

The result shows different levels of development of necrosis on the different hybrids of clones and parentalclones. The pathogen was then isolated and grown in a Petri dish, and its sporangia observed (fig. 2). The least tolerant (t) had the highest scores while the most tolerant had the smallest scores. Different individuals of the hybrids of clones and clones manifested different levels of development of necrosis ranging from very low (t=0.79) to average (1.47 ≤ t ≤ 3.75) to severe (t ≥ 3.79). Genotypes which manifested weaker development of necrotic symptoms (t ≤2.0) represented tolerance while those with significant levels (t ≥4.79) of development of necrosis were likened to be tolerant individuals.

After inoculation, the manifestation of the symptom varied from one progeny to another and from one clonal group to the other. Some of the hybrids of clones had only a few localized necrotic spot, others had necrotic spot that coalesced and others were severely affected with the visible presence of spores produced.

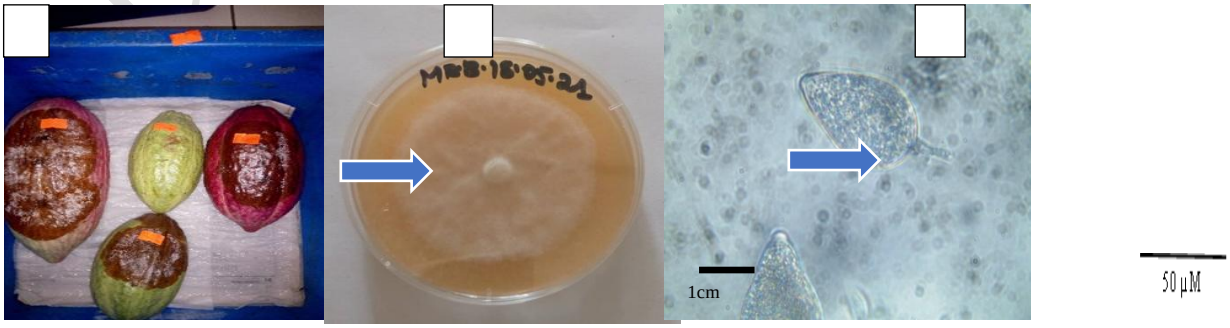

Fig. 2: Culture of *P. megakarya* and observation of its sporangiospores (A: Necrotic cocoa pods, B: Clusters of mycelium in a Petri dish, C: Sporangiospores)

3.1.2 Tolerance of hybrids of clones

Analysis of variance (ANOVA) was carried out to determine the level of significance of the tolerance between the hybrids of clones.

In the analysis of variance, all the values of probability P obtained is 0.01 at the 5% threshold of the Student Newman-Keuls multiple comparison test (Table 1). The value obtained from the Fischer test, calculated F, is 3.12. Therefore, there is significant difference in the tolerance of the 5 hybrids of clones. Appendix 1 contains the data sheet.

The figure 3 is a graphical presentation of the means of the tolerance of the 5 hybrids of clones to the black pod disease tested with the EBWL 1-289 of *P. megakarya* that was confirmed to be moderately virulent. The ANOVA on table 5shows that there was a significant effect (P=0.01) in the mean tolerance of the hybrids of clones to the *P. megakarya*. The progeny PA 107 X SNK 614 was the most tolerant of the five hybrids of clones and had the smallest values of tolerance (0.79) and the progeny T 79/501 X SNK 64 with an average tolerance of 2.00 the least tolerant.

Table 1: ANOVA of tolerance level on the detached pods of 5 hybrids of clones.

Source	**df**	**SS**	**MS**	**F**	**Pr > F**
Progeny	4	19.839	4.959	3.12	0.017
Error	113	179.525	1.588		
Corrected Total	117	199.364			

df: degree of freedom; SS: sum of the squares of the deviations; MS: mean square; F cal: calculated Fischer value; P: Probability; 0.01: significant

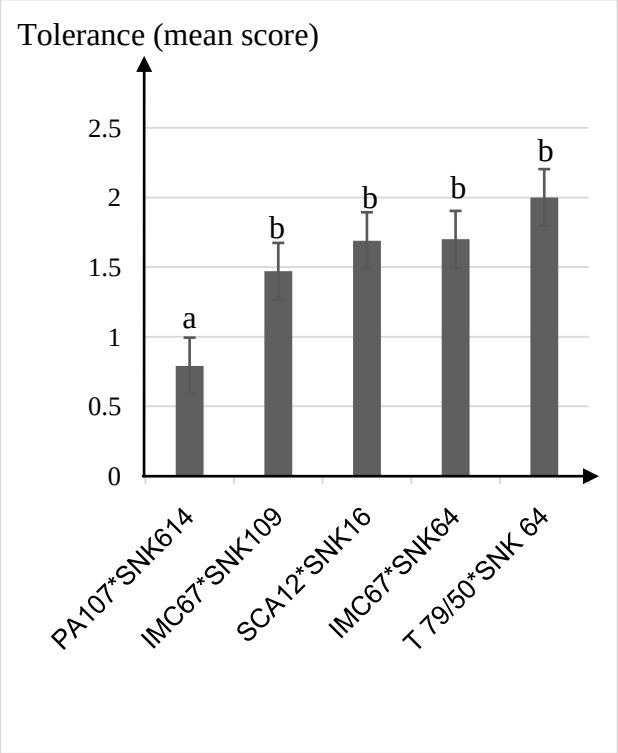

Fig.3: Comparison of the mean score of tolerance of hybrids of clones to *P. megakarya.*

3.1.3 Ranking of hybrids of clones

Statistical analysis reveals that for the five (5) hybrids of clones there are significantly different levels of tolerance (p=0.01) (Table 2). The score for each progeny was calculated by averaging scores of all individuals in the progeny.Following the separation of the means carried out using the Student Newman-Keuls test, 2 statistical groups were counted for the 5 progeny that constituted the study population. With an average score of 0.79, the PA107*SNK614 progeny is the most tolerant population in the study, while T 79/50*SNK 64 is the most sensitive with an average score of 2.00. All 5 hybrids of clones had scores less than or equal to 2.00. However, the variation of individual scores from one progeny to another is not uniform (Table 6). The greatest interval between the lowest score (most tolerant) and the highest score (most sensitive) is observed in the IMC67*SNK64.

Table 2: Ranking of the hybrids of clones according to average level of tolerance to *P. megakarya*.

Progeny	**Mean Score**	**SNK Group**
T 79/50*SNK 64	2.00*	A
IMC67*SNK64	1.7	A
SCA12*SNK16	1.69	A
IMC67*SNK109	1.47	A
PA107*SNK614	0.79	B

*: The hybrids of clonesare ranked from the least tolerant to the most tolerant

3.1.4 Tolerance of the clonal groups

To determine the level of significance of the tolerance level of the clonal groups, analysis of variance (ANOVA) was conducted.

In the analysis of variance, all the values of probability P obtained is less than 0.0001 at the 5% threshold of the Student Newman-Keuls multiple comparison test (Table 3). The value obtained from the Fischer test, calculated F, is 7.92. Therefore, there is significant difference in the tolerance of the 6 clonal groups used in the study. Appendix 1 contains the complete data sheet.

The figure 4 presents the graphical representation of the tolerance of the clonal groups to the black pod disease caused by *P. megakarya*. The different clonal groups have different mean scores which are represented by the height of the bars on the histogram. The analysis of variance equally showed that was a very strong significance at P=0.001. However, the IMC clonal group was most tolerant the black pod disease. Though there was a variation in the tolerance among the clonal groups, the SNK clonal group was the most susceptible group with a mean score of 5.07.

Table 3: ANOVA of tolerance level on the detached pods of 6 clonal families.

Source	**df**	**SS**	**MS**	**F**	**Pr > F**	
Family	5	141.2234	28.2446	7.92	<0.0001	***
Error	170	606.0890	3.5652			
Corrected Total	175	747.3125				

df: degree of freedom; SS: sum of the squares of the deviations; MS: mean square; F cal: calculated Fischer value; P: Probability; <0.0001: highly significant.

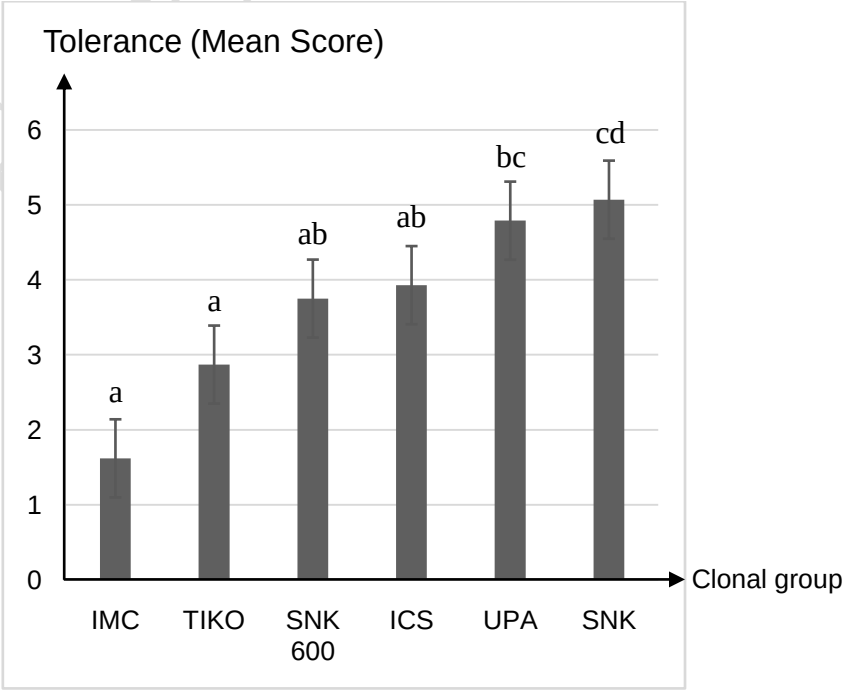

Fig.4: Comparison of the means of the tolerance of the clonal groups to the black pod disease

3.1.5 Ranking of the clonal groups

Statistical analysis reveals that for the six (6) clonal groups there are significantly different levels of tolerance (p=0.0001) (Table 4). The score for each clonal group was calculated by averaging scores of all individuals in the Clonal group.

Table 4: Ranking of 6 clonal groups according to their tolerance to *P. megakarya*

Clonal Group	**Mean Score**	**SNK Group**
SNK	5.07	A
UPA	4.79	A
ICS	3.93	AB
SNK 600	3.75	AB
TIKO	2.87	BC
IMC	1.62	CD

3.1.6 Tolerance of the hybrids of clones and the clonal groups

ANOVA was conducted to determine the level of significance of the tolerance between the hybrids of clones and the clonal groups.

In the analysis of variance, all the values of probability P obtained is less than 0.0001 at the 5% threshold of the Student Newman-Keuls multiple comparison test (Table 5). The value obtained from the Fischer test, calculated F, is 23.39. Therefore, there is significant difference in the tolerance of the 6 clonal groups used in the study.

The results of the tolerance of the hybrids of clones and the clonal groups are present in the figure 5 below which shows a variation the mean tolerance from 1 group to the other.

The figure 5 above presents the graphical representation of the tolerance of hybrids of clones and clonal groups to the black pod disease caused by *P. megakarya.* The mean scores vary form one progeny to another and from one clonal group to the other. The height of the bars represents the average score of the tolerance to the black pod disease. Though the hybrids of clones had varying levels of tolerance, they all had mean values less than or equal to 2. The clonal groups on the other hand were averagely tolerant to susceptible. They had mean values ranging from 2.87 to 5.07 on a 0-8 rating scale. The IMC clonal group showed more tolerance than the hybrids of clones; SCA 12 X SNK 16, IMC 67 X SNK 64 and T79/501 X SNK 64. The SNK clonal group was the most susceptible group.

Table 5: ANOVA of the level of aggressiveness of the *P. megakarya.*

Source	**df**	**SS**	**MS**	**F**	**Pr > F**	
Progeny	37	1190.159864	32.166483	23.39	<.0001	***
Error	256	352.071429	1.375279			
Corrected Total	293	1542.231293				

df: degree of freedom; SS: sum of the squares of the deviations; MS: mean square; F cal: calculated Fischer value; P: Probability; <0.0001: highly significant.

Tolerance (Mean Score)

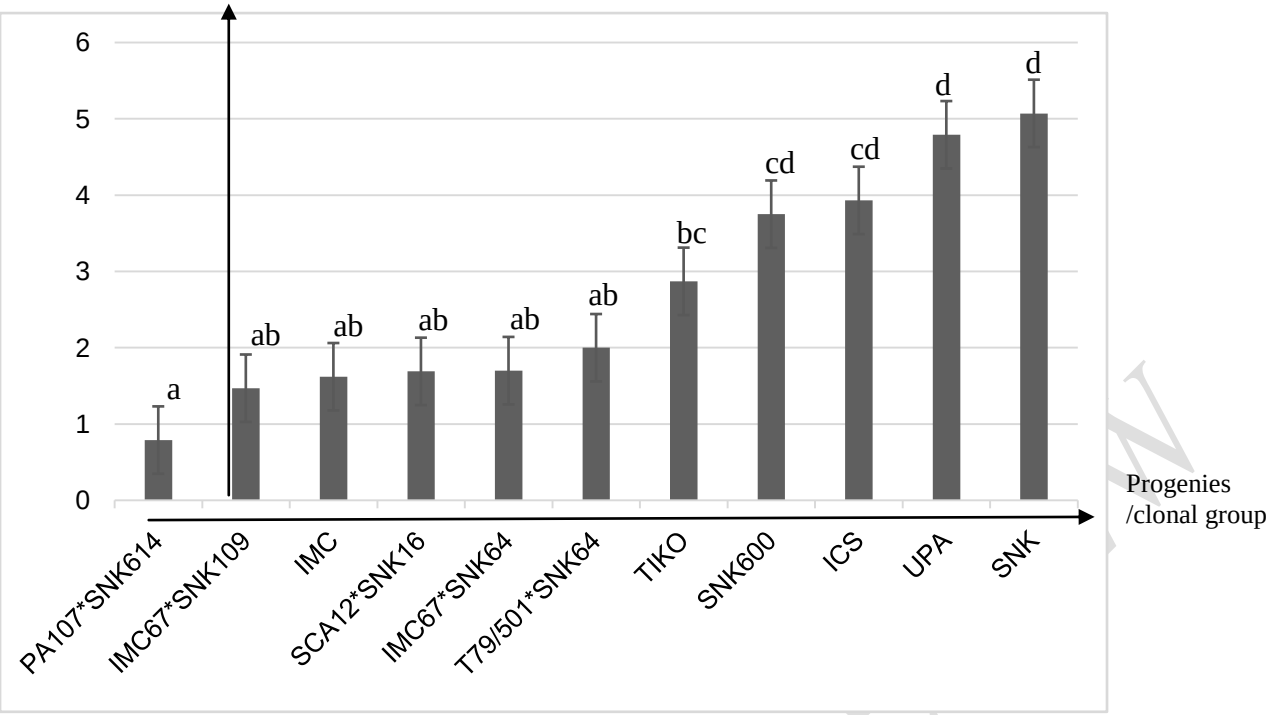

Fig.5: Tolerance of hybrids of clones and clonal groups to the black pod disease

3.1.7 Tolerance of Hybrids of clones and clonal groups

Following the separation of the means carried out using the Student Newman-Keuls test, 5 statistical groups were counted for the 5 hybrids of clones and 6 clonal group that constituted the study population. The least score (0.79) was obtained from the progeny PA107*SNK614 which constituted the most tolerant population in the study. On the other hand, the SNK clonal group was the most sensitive with an average score of 5.07. All of the hybrids of clones were ranked as tolerant with scores ranging from 0.05 to 3.0 and 40% of the hybrids of clones were more tolerant the most tolerant clonal group (IMC) clonal group.

The table 6 below ranks the hybrids of clones and the clonal groups according to the average level of tolerance obtained at the end of the tests (average of the three replicates over time).

Table 6: Ranking of the hybrids of clones and the clonal groups

Progeny / Clonal Group	**Score**	**Rank**
PA107*SNK614	0.79	A
IMC67*SNK109	1.47	AB
IMC	1.62	AB
SCA12*SNK16	1.69	AB
IMC67*SNK64	1.70	AB
T79/501*SNK64	2.00	AB

TIKO	2.87	BC
SNK600	3.75	CD
ICS	3.93	CD
UPA	4.79	D
SNK	5.07	D

3.2 Discussion

3.2.1 Virulence of Phytophthora isolates and the resistance mechanism of cocoa.

When the conditions are right, *P. megakarya* grows through the mycelium, thus when cultured in media under lab conditions the *P. megakarya* isolate had optimum growth and produced mycelium. This is similar to the results obtained by[29], who reported that there were optimum mycelia growth of *P. megakarya* at 25ºC.Light, temperature and humidity have a considerable influence on the growth and evolution of *P. megakarya.* Light inhibits itsgrowth while low temperatures and high relative humidity promotes its growth, thus infection due *P. megakarya*is likely to be low especially on farms where shade and canopy management are effectively managed.[28] equally reported that when the relative humidity is high the *P. megakarya*will infect the cocoa pods and cause disease. According to[20], light inhibits the growth of *P. megakarya* and light intensity is related to temperature. Thus, infection caused by *P. megakarya* is said to most likely to be lower if the shade is properly managed.[4], reported that when the relative humidity during the day remained higher above 80% under the tree canopy with rain fall and low temperature black pod will develop faster. To detect the pathogen, molecular techniques are faster, more specific and accurate, than other methods[11]. Thus, the use of molecular tools also makes it possible to gain an insight at the molecular level to the processes which underlie pathogenesis [19].

3.2.2 Artificial inoculation

The use of artificial inoculation of *P. megakarya* in this study makes it possible to identify variations in the levels of tolerance that exist between different hybrids of clones. The individuals of the clonal groups and the hybrids of clones each showed different levels of tolerance or sensitivity marked by the development and spread of necrotic lesions on the cocoa pods testedas reported by[40]. The appearance of necrotic lesions observed on the infected detached pods is due to the presence of the mycelium of *P. megakarya*on the pod. These studies are similar to those obtained by [34] and [43] that reported varying levels of infection (necrotic lesions) on cocoa pods and leaves caused by *P. megakarya*. Detection of the level of tolerance and susceptibility of a genotype to the black pod disease in the early stages of the plant's growth is possible using the leaf disk test, [34] who reported the use of the leaf disk test to detect the early resistance of cocoa genotypes to the black pod disease. Similarly mature unripe pods are used for the detection of the tolerance to the disease. This technique was used for the testing of hybrids of clones and clones.The tolerant individuals were differentiated from susceptible individuals through the appearance of necrotic lesions.These results are

similar to the results obtained by[22]that showed that cocoa pods from different genotypes had variations in the level of evolution of necrotic lesions which he described as an indication in the tolerance to the black pod disease.

3.2.3 Mechanism of tolerance to the black pod disease.

The resistance of cocoa to *Phytophthora* operates at two distinct stages: penetration stage which restricts the entry and establishment of the pathogen (this reduces the number of lesions and it occur on the surfaces of the pod) and the post-penetration stage, which reduces the rate of spread and expansion of the pathogen[22]. The first barrier by plant pathogens during infections are mainly cuticle and cell wall[3], which in cocoa are found in the pod husk. This implies that the thickness and the composition of pod husk play important role in disease infection and severity. In studies by[33], genotypes of cocoa with thicker pod husks showed more tolerance to the black pod disease than genotypes with thinner pod husk. Pod surface waxes have also been reported to play an important role in the defense against the development of black pod disease and genotypes with higher amount of wax on the pods and leaves were found to be more tolerant to black pod than cocoa genotypes with lesser amount of wax when tested using the detached pod tests and the leaf disk tests[32].

[21] suggested that if host plants secrete compounds that inhibit spore adhesion (occurring roots, leaves and pods), then it is likely that the rate of successful infection would be reduced because a successful *Phytophthora* infection cycle begin with the firm adhesion of motile zoospores to the host tissue. Therefore, during the breeding for resistance, the use of genotypes with thick pod husks may significantly minimize the susceptibility to the black pod disease. This can equally delay or resist *Phytophthora* penetration and will significantly minimize disease initiation in the unwounded pods.Moreover, cocoa hybrids with thick pod husks, will have fewer tendencies to be injured by animals, pests, man etc. as injuries results in a breakdown of resistance at the penetration stage (pod husk surfaces), rendering the pod highly susceptible to black pod.[41]reported that the defense gene polymorphism was responsible a genotypes capacity to tolerate infection by the *Phytophthora* spp. according to the finding of their study the PR-1 gene bind steroids and cause pathogen membrane linkage. As such a delay in lesion development which leads to subsequent delays sporulation will lead to a reduction of disease epidemics.

3.2.4 Tolerance within and among hybrids of clones

The results of the detached pod test of the hybrids of clones showed a high to moderate tolerance to the infection by *P. megakarya*. The PA107*SNK 614 hybrid showed a high level of tolerance to the black pod disease, this result corroborates with that reported by[16]that ranked the hybrid PA107*SNK 614 as productive and tolerant to the black pod disease.The progeny IMC67 * SNK109also showed high tolerance to the black pod disease.Both parent clones have some levels of tolerance to the black pod disease, with the IMC clonal group being tolerant while the SNK 109 is averagely tolerant, as reported by[34]. Hybrids of clones will manifest hybrid vigor as a result of the ability of parental clones to combine during crossing[44]. No immunity was observed in all the hybrids of clones, but the hybrids of clones had disease rating scores that were less than 2.5 corresponding the rating of the SCA 6 which is the control clone for tolerance to the black

pod disease, these results are similar to those obtained by [23].[47]also reported that the most of the hybrids produced had scores ranging from tolerant to moderately tolerant.

The ANOVA carried out on the evolution of the necrosis on the different individuals of the genotypesshow a significant hybrid effect (P=0.001). There was a great variability in the level of infection as manifested on the detached pods as some individuals of the same progeny had more lesions than others during the test.This varying rates of evolution of the necrotic lesion can be said to be caused by the variations in the genotypes. These results are similar to those obtained by[26] that showed a great level of heritability of the tolerance of hybrids of clones to the black pod disease manifested by the variation of the sizes of necrotic lesions on the pods of the hybrids of clones which were reduced compared to that of the parent clones.

The comparison of the performances of the individuals of the 5hybrids of clonesfrom the average score of the evolution of the necrosis on detached pods makes it possible to say that there is a significant difference between the performances of the hybrids of clones. The existence of an individual effect within each progeny confirms the different reactions of the individuals of the same progeny [46].[34]equally reported that hybrid families showed a highly significant plant effect when tested using the leaf disk test.

By using the Student Newman-Keuls test to separate the individuals within the genotypes by use of their mean whose values was indicative of the transmission of tolerance [44], 3 statistical groups (A, AB and B) were obtained. There is therefore a variation in tolerance within the hybrids of clones. This variation in tolerance measured by the detached pod test can be used for the selection of cocoa trees. This resultis similar to what was demonstrated by [15]using the leaf disk test.

[16]demonstrated that three series of inoculations over time are sufficient to give a fairly precise indication of the level of tolerance of a clone (parent) or of a hybrid of clones (offspring). This supports the results obtained in the experiments.

The parent clones used as progenitors for the creation of hybrids of clonesused in this study were chosen because of their good level of tolerance to *P. megakarya*. The results obtained at the end of the artificial inoculation tests on leaf discs show a good level of tolerance in all the hybrids of clones studied. Thus, the average score varies from 0.79 in the most progeny (PA 107*SNK 614) to 3.00 in the most susceptible progeny(IMC 67*SNK 64).This result is an indication of the transfer of tolerance of the black pod disease similar to results obtained by[9].

3.2.5 Tolerance between hybrids of clones and clonal group

The clone groups showed different levels of development of necrosis when infected with the *P.megakarya* inoculum. These results ranged from moderately tolerant to susceptible, this is similar to the results obtained by [45] and [24] stating that they will be a variation in the level of necrosis on cocoa pods caused by the *P. megakarya*and this is accounted for by the variation of the varieties. According to [44]the heritability is influenced by presence of peroxidase.Only the IMC clonal group (of all the 6 clonal groups tested) that showed more tolerance than 3 of the 5 hybrids of clones tested (SCA 12*SNK 16, IMC 67*SNK 64 and the T79/501SNK 64). This result tallies with the findings of [37] which suggested that a new hybrid population be created using progenitors which have shown tolerance to *P. megakarya.*

The ANOVA carried out on the evolution of the necrosis on the different hybrids of clones and the clone groups was highly significant at (P=0.0001). This explains the high variation that exist in the levels of tolerance between the individuals of the hybrids of clones and those of the clonal group. And the hybrids of cloneswas highly to moderately tolerant while the clonal groups ranged from moderately tolerantto susceptible (the SNK group).

The SNK group was the most susceptible sub group in the study. The scores ranged from 2.62-8.00 with an average score of 5.07. The individuals of the UPA clonal group had scores ranging from 3.87-5.37 and average score of 4.79, and was ranked in the same group and the SNK group.The ICS and the SNK 600clonal group were placed in second group and had scores ranging from 2.12-5.75 and 3.12-5.00 and mean scores of 3.93 and a 3.75 respectively. The individuals of the TIKOclone group had scored a score 2.87 and 1.62 respectively and were placed in 2 separate SNK Groups, this is similar to the results obtained by [16] in which the SNK clones susceptible to the black pod disease while the SNK 600 were tolerant.

On the other hand, the hybrids of clones had 2 groups: SCA 12*SNK 16 with scores between 1.50-3.00 and mean score 1.69, IMC 67*SNK 64 with scores 0.87-3.00 and mean score 1.70, T 79/501*SNK 64 with scores between 1.25-1.75 and mean score 1.47 and PA 107*SNK 614 with scores 0.50-1.12 and mean score of 0.79.[16] had similar results when the progeny was tested for the tolerance to the black pod disease, he equally reported that the progeny PA 107*SNK 614 was also very productive. Upper Amazonian Forastero are known to harbor a good source to the tolerance to the *Phytophthora*sppand that hybrids of clones with Upper Amazonian Forastero genes were averagely more tolerant[48] and [27]. [13] reported that parent clones had the ability to combine and produce tolerant and vigorous hybrids of clones. [42] equally reported that parent clones had the capacity to transfer their potential to tolerate the back pod disease to their offspring (progeny).

4. CONCLUSION

The main objective of the study was to assess the tolerance to *P. megakarya*on 5 cocoa (*T. cacao*) hybrids of clones developed by IRADcocoa. Tolerance within these hybrids of clones was measured using Iwaro's detached pod test. Some of the parent clones were also evaluated alongside the hybrids of clones. The results obtained showed varying levels of tolerance to the disease for the different clones and hybrids of clones.Forty percent (40%) of the hybrids of clones were more tolerant than the most tolerant Clonal group. Although the average tolerance score varied from one progeny to another, the results obtained showed that all the hybrids of clones were tolerant to the back pod disease caused by the *P.megakarya*, which is the major course of yield loss in all producing areas especially in Cameroon.These results are just an indication of the real potential of the hybrids of clones. Thus, more research needs to be done both in the laboratory and on the field. The evolution of the disease on the field should be done for every genotype of all the hybrids of clones created in this collection. These phenotypic data be used alongside molecular techniques such as genomics for a better comprehension of the potentials of the genotypes and provide guidance for a more accurate selection. Through this the potentials of the hybrids of clones will be better understood. This is because the tolerance manifested can be as a result of the pod husk thickness, epicuticular wax or inherent genetic factors and this could can account for the variation in the number and size of the necrotic lesions caused by the *P. magakarya*. These properties of the pods can be used as markers traits to select for the resistance to the black pod disease using molecular techniques.

The variation observe between the clonal groups and the hybrids of clones is indicative of the transfer of the traits of tolerance from the parent clones to the hybrids of clones. All the hybrids of clones showed a higher level of tolerance than the clonal groups, with the exception of the IMC clonal group was more tolerant then 3 of the 5 hybrids of clones assessed. This tolerance can further be exploited in the creation of other hybrids for the tolerance to the black pod disease. Though the other hybrids of clones had less tolerance than the IMC clonal group, their tolerance was moderate, which makes them suitable for use in clonal farms as tolerant varieties. Due to the variation in the tolerance levels of the individuals withing the same progeny, farmers can propagate (vegetatively through grafting and budding or grafting) the individuals to have best results in their fields.

The ranking of the hybrids of clones and the clonal groups showed that the hybrids of clones were more tolerant to the black pod disease. This ranking serves as an important tool for the selection of an alternative progeny in in terms of the tolerance to the black pod disease. This information can be of use to IRAD as the researchers continue the research process on these hybrids of clones. The farmers this will serve as a guide of what material to ask for when extending their plantation, creating new plantations or the regeneration of old and unproductive plantations.

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