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Analysis of the resistance to *Phytophthora* pod rot within local selections of cacao (*Theobroma cacao* L.) for breeding purpose in Cameroon

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The level of resistance against *Phytophthora* pod rot (PPR) was assessed among locally-selected accessions of cacao, the so-called selection of Nkoemvone (SNK), in Cameroon. The evaluation approaches have included four tests: Leaf disc inoculation (LDT); detached pod inoculation (DPT); artificial inoculation of pods and counting of successful infections (%SI); and field observations of the disease incidence (FI). The studied accessions were classified into four groups according to their genetic origin: SNK; SNK600-Tr (SNK*ICS); SNK600-Tr*UA (UPA*ICS); SNK600-Tr*UA (UPA*SNK). In all four testing methods, the groups UPA*ICS and SNK appeared respectively as the least and the most susceptible accession groups. Four resistance classes were defined for each testing method. A great part of the accessions were found in the intermediary resistance classes, based on DPT, LDT and %SI. Few accessions have displayed a significant level of PPR resistance in all testing methods. Rank correlation of the different accessions groups based on their performance in the four tests have shown that all correlation coefficients were positive (r = 0.37 – 0.87) and significant ($p \leq 0.06$). The best correlation was registered between %SI and FI with r = 0.87 (p = 0.01). Subsequently, a unique classification of all accessions was done according to the scores recorded in all the testing methods. The use of locally-selected accessions (SNK) in the varietal development as well as in the cacao seed production is discussed.

Key words: Cacao plant pathology, oomycetes, screening methods, resistant cultivars, selection.

INTRODUCTION

Cacao (*Theobroma cacao* L.) is a perennial crop of high economic importance in several cocoa-producing countries, including Cameroon. However, cacao cultivation is threatened by many constraints, such as the *Phytophthora* pod rot (PPR) disease. In absence of any chemical control, cacao pod losses may reach 90 to 100% (Despréaux et al., 1988), posing to the ongoing research the need to find out resistant cultivars. In 1950s, field surveys were carried out in existing cacao farms of southern Cameroon in order to identify high yielding cacao trees (Braudeau et al., 1952). The outcomes of these surveys were the selection of two main populations belonging to the two major genetic groups of cacao: the Trinitarios (Tr) and the Lower Amazon Forasteros (LAF). The pod size was the first criterion of selection. Therefore, most of the LAF known to bear pods with limited size (Bartley, 2005) failed to be selected. The selected trees were transferred and multiplied on the

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research station of Nkoemvone and thus identified using the prefix selection of Nkoemvone ('SNK'). All the SNK accessions were subsequently used to create open-pollinated crosses. As part of the breeding program, all these accessions planted in clonal trials were assessed based on the following criteria: vigor; yield; number of beans per pod; bean size and weight. The best ones were maintained in so-called clone collections. Base on their genetic traits such as yield, some of the SNK cultivars were used as progenitors in seed gardens. Unfortunately, no assessment of the level of tolerance to *Phytophthora* was performed on these clones before their use as progenitors. This resulted in a rather high level of susceptibility of the progenies released to farmers.

Progress in breeding has been facilitated by effective screening methods to identify genotypes with superior abilities to be incorporated into breeding programmes. Generally, there are two types of pathology tests to evaluate PPR resistance: *in vitro* early screening tests on leaves (Nyassé et al., 1995) and on detached pods (Iwaro et al., 2000), *in vivo* screening tests on pods attached to the cacao tree (Blaha and Lotode, 1976) and field evaluation of the natural infection (Ndoumbè et al., 2002). At the end of 1970s, an artificial inoculation test was performed on pods yet attached to the trees, followed by the counting of pods showing symptoms 10 days after inoculation (Blaha and Lotodé, 1976). This test was used in order to assess 112 genotypes, among which 78 clones were locally selected (SNK). The data obtained from this test showed a high level of susceptibility to *Phytophthora* for most of the clones used as progenitors in the commercially released progenies. This test was then used to assess the level of tolerance to *Phytophthora* of trees in local progeny trials and this study resulted in the selection of 42 genotypes showing a very low proportion of infected pods after artificial inoculation. These genotypes were cloned, named as SNK 600 to SNK 641, and grouped based on their different genetic origins: (i) full-sib progenies issued from crosses between two Trinitario parents, (ii) crosses between one Trinitario and one Upper Amazon Forastero parent, and (iii) selfing of SNK 64.

Later on, Berry and Cilas (1994) compared the pod inoculation data obtained from six clones with the level of natural infection observed on these clones in the field, and failed to find any significant positive correlation. On the other hand, they noticed that clones with large pods were more heavily infected in natural conditions than those with small pods and suggested that pod volume might be a major factor in natural infection. During the last 15 years, efforts have been made to develop new tests in order to assess the level of resistance of cacao to PPR. A first test, based on lab artificial inoculation of leaf discs followed by a scoring of the symptoms, was developed in the mid 1990s by Nyassé et al. (1995) and, later on, a test based on pod inoculation in the lab, followed by scoring symptoms was developed by Iwaro et al. (2000), in order to select progenitors combining high yield and resistance to PPR.

Selection of resistant material in the field and all the known tests for resistance based on pod inoculation necessitates waiting until a tree bears pods before its level of resistance to black pod infection can be determined. These methods, therefore, do not offer a quick means of breeding for resistance. Consequently, tests at the seedling stage that provide information about pod resistance are earnestly needed to save time and cost. Few reliability studies were carried out to confirm the validity of those screening tests have shown positive correlation between pod and leaf tests (Tahi et al., 2000; Nyadanu et al., 2009). However, the correlation between the reactions of detached leaves and pod tests to *Phytophthora* sp. infection is not well understood and utilized. Wheeler (1992) emphasized the need to understand the relationship between pod resistance and leaf resistance to PPR and, the relationship between detached leaves and pod tests infection in the field to expedite the screening of germplasm. With the objectives of verifying the screening power of four assessing methods towards identification of the best PPR-resistant genotypes among the SNK collection, the present paper shows the data obtained on the levels of PPR resistance for the SNK clones, including those selected in local farms (SNK 1 to 599) in the 1950s, and those from local progeny trials in the late 1970s (SNK 600 to SNK 641).

MATERIALS AND METHODS

Cacao accessions

A total of 105 accessions available in the clone collections of the Nkoemvone IRAD Station (Southern Cameroon) was considered in the study. The accessions were classified into two groups, according to their role in the cacao breeding program in Cameroon (Table 1):

(1) The SNK group (72 accessions) labeled from SNK 1 to SNK599, selected in the 1950s at local farms for their yield levels.
(2) The SNK600 group (33 accessions) composed of cloned progenies and subdivided as follows, according to their genetic origin:
(i) The SNK600-Tr group (13 accessions) originated from crosses between a SNK clone (SNK 1 to SNK 599) and an ICS clone (from the 'Trinitario' genotypes introduced from Trinidad and Tobago in the early 1960s).
(ii) The SNK-Tr*UA group (20 accessions) originated from crosses between a Trinitario clone (SNK 1 to SNK 599 or ICS) and an Upper Amazon Forastero clone.

Screening tests for resistance to PPR

Leaf disc inoculation test (LDT)

An early screening method (Nyassé et al., 1995) based on a leaf disc inoculation tests and a disease rating was used in the study. All the plant material was inoculated with a moderately aggressive isolate of *Phytophthora megakarya* collected in farmers' field and

Table 1. List of the locally-selected accessions used in the study and available in cacao genebank at Nkoemvone (Southern Cameroon).

Genetic group	Accession groups	Progenitors	Corresponding accessions
Tr	SNK-Tr[1]	-	SNK 7 10 12 13 15 16 17 27 29 30 31 32 33 35 37 46 48 52 64 67 109 111 141 181 203 213 223 267 269 277 322 332 343 344 348 353 361 376 377 392 404 413 415 416 417 422 443 444 450 451 452 456 459 460 461 467 469 476 478 479 480 485 488 490 496 500 501 502 503 504 505 506
	SNK600[2]-Tr	SNK*ICS (SNK13*ICS95; SNK30*ICS95; SNK416*ICS95)	SNK 600 602 603 604 605 606 607 608 609 610
		ICS*SNK (ICS43*SNK37; ICS84*SNK37; SNK416*ICS95)	SNK 611 612 613
TrxUA	SNK600-TrxUA	UPA*ICS (UPA134*ICS1; UPA143*ICS95; UPA143*ICS43;UPA134*ICS43)	SNK 614 615 616 618 619
		ICS*UPA (ICS84*UPA337)	SNK 620 621 622 623 624
		UPA*SNK (UPA134*SNK64; UPA143*SNK64)	SNK 625 626 627 628 629
		SNK*UPA (SNK30*UPA337; SNK16*UPA143; SNK109*UPA143)	SNK 630 631 632 633 634

[1] Trinitario clones selected from farmers fields in the South of Cameroon in the 1960s and transferred to the genebank of the IRAD Nkoemvone Research Station (Southern Cameroon), [2] So-called SNK600 series of clones, they were selected on-station in UAxTr and in TrxTr crosses.

maintained in the Plant Pathology Laboratory at IRAD Nkolbisson Centre (Cameroon). Prior to their evaluation with the leaf disc inoculation test, all the studied cacao accessions existing in the field as adult trees (ages above 30 to 40 years) were duplicated in the nursery using a vegetative propagation technique (top-grafting on 6-months rootstocks).

Three inoculations series were carried out for each accession tested and the interval between each one was at least 30 days. In each inoculation series (replicate), two or three leaves of approximately two months old from non-lignified twigs as described by Nyassé et al. (1995) were collected early in the morning from relatively flexible branches (green turning to brown) of each accession. Only the synchronized flushes of all the plants were used at the same time for a single replicate. Thirty leaf discs of 15 mm diameter were cut from those two or three collected leaves per accession in the afternoon and placed upside down in three wetted plastic trays, with 10 discs per tray. The covered trays were incubated in the darkness overnight at approximately 25°C. Inoculations were carried out the next morning with a 10 µl suspension of *P. megakarya* calibrated at 300,000 zoospores per ml. Symptoms (disease scores) were observed five days after inoculation by using a 5 point scale, with 0 = no symptoms; 1 = very small localized penetration points; 2 = small penetration spots, sometimes in a network; 3 = coalescing lesions of intermediate size; 4 = large coalescing brown patches; and 5 = uniform large dark brown lesions.

Detached pod test **(DPT)**

The resistance of cacao accessions was also assessed according to the method developed by Iwaro et al. (2000). Half of the pods surface was sprayed at a distance of 30 cm, using an atomizer containing a zoospore suspension of the same isolate used with the leaf inoculation test. For each accession, three inoculation rounds were carried out at different dates and ten pods were inoculated during each round. After four days of incubation, the inoculated pods were assessed based on the frequency and size of the lesions formed. The severity of infection was rated on an 8-point scale as follows: 1 = No symptom, highly resistance to penetration; 2 = 1-5 localized lesions, resistant; 3 = 6-15 localized lesions, moderately resistant; 4 = >15 localized lesions, partially resistant/resistant to spread of lesions alone; 5 = 1-5 expanding lesions, partially resistant/resistant to penetration alone; 6 = 6-15 expanding lesions, moderately susceptible, 7 = >15 expanding lesions, susceptible; 8 = coalesced lesions, highly susceptible.

Field tests

Percentage of successful infections (% SI) on attached pods

This method was developed by Blaha and Lotode (1976). It has the advantage to be closer to what happens naturally in the field. The tests are carried out on living pods attached to cacao trees, and therefore differ from the previous testing methods (LDT and DPT) that are undertaken under laboratory conditions. Fruits (pods) are usually tested during heavy rainy seasons, corresponding to the peak of PPR natural infections. Cacao trees in the field experiments are maintained in natural conditions, with no fertilizers and pesticides applications. Attached pods are inoculated with zoospores of *P. megakarya* on any part of the husk. In this work, the evaluation of symptoms was monitored continuously during twelve days. The resistance to the penetration of the pathogen was assessed by counting the number of pods successfully infected. The average number of pods infected per accession varied between 50 and 200. The rate of infection for each accession (% SI) was estimated by the ratio between the numbers of successful infections and the total number of pods inoculated. A total of 61 accessions were tested.

Field incidence (FI) of PPR

The natural infection in the field was measured during the period of PPR incidence. This period corresponds to the start and the end of the rainy seasons within a year. The evaluation of PPR incidence for each accession consists on the counting of both the number of PPR-infected and the healthy pods during the campaign. Pods that were rotten (affected by PPR) wilted (early desiccation of a physiological nature) damaged by rodents, and ripe and healthy, were counted weekly. During the counts, sanitation harvesting was carried out: all pods except unripe healthy pods were removed. Losses caused by PPR were estimated in relation to potential production (excluding rodent-damaged pods) using the formula giving the pod rot rate per tree (Rrot):

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

Statistical analyses

The data collected were analyzed with SAS software version 8 (2000). ANOVA was computed for the results obtained in all testing methods and the data was tested for adjustment to the major assumptions of ANOVA analysis. The Newman and Keuls (Cochran and Cox, 1957) test at 5% probability level was used to compare means of individual accessions and means of groups of accessions. Four resistance classes were designed in the four testing methods: class A (resistant); class B (moderately resistant); class C (moderately susceptible); class D (susceptible). Each accession was assigned to one of the classes according to their average disease score. Subsequently, the overall level of resistance of each accession was determined by compiling the performance recorded in each of four testing methods as follows: Susceptible (‘S’) = C or D in all the testing methods; Moderately Susceptible (‘MS’) = more than 50% of C or D in all the testing methods; Moderately Resistant (‘MR’) = maximum 50% of C or D in all the testing methods; Resistant (‘R’) = A or B in all the testing methods. Spearman rank correlation (1904) was used to calculate the correlated coefficients at their associated probabilities among the four different testing methods of the study.

RESULTS

Analysis of PPR resistance for each testing methods

Leaf disc test (LDT)

The average disease score (DS) varied between 2.48 (UPA*ICS) and 3.27 (SNK) (Table 2). Considering the four resistance classes of the LDT, only the accessions group UPA*ICS was found in the class B (moderately resistant), the three other ones were all grouped in class C (moderately susceptible). In the SNK group, about 24.5 and 62.3% accessions were grouped respectively in B and C resistance classes. The same classes B and C were the most representative in SNK*ICS and UPA*SNK groups, with 18.2 and 72.7% (SNK*ICS), and 25.0 and 50.0% (UPA*SNK) respectively. In the most resistant group UPA*ICS, all the accessions were found in one class (B). The lowest DS were recorded in three resistant accessions: SNK478 (DS=1.6) and SNK111 (DS=1.7) in SNK group; SNK630 (DS=1.3) in UPA*SNK group. Even if two of the three resistant accessions were identified in the SNK group using LDT, the five most susceptible ones were also registered in the same group (SNK 376; 267; 52. 416; 10) with DS ranging from 4.1 to 4.5 (class D).

Detached pod test (DPT)

Following the mean score of their corresponding accessions, the four groups displayed different levels of resistance in this test (Table 3). The SNK group was the less resistant against PPR with an average DS of 5.6, followed by the UPA*SNK group (DS = 5.56, not significantly different to SNK AG at α = 5%). The UPA*ICS group was less susceptible (DS=4.60). The accessions were distributed in different resistant classes according to their average disease score. In the SNK group, most of these accessions belong to the moderately susceptible (47.7%) or the susceptible class (40.0%). In the groups SNK*ICS and UPA*SNK, the most susceptible classes have also registered the great number of accessions. In contrast, half of the accessions were found in the moderately resistant class. No accessions were grouped in the resistant class (class A) in UPA*ICS and UPA*SNK groups.

Successful infection rate on attached pods (%SI)

Amplitude of average DS of the four accessions groups was high. The DS values varied from 11.6 (UPA*ICS) to 77.75 (SNK) (Table 4). However, three out of the four accessions groups belonged to the same resistance class (A). Except for SNK 490; 413 and 64 in SNK group, all the other accessions were classified either as moderately susceptible (class C, 28.3%) or susceptible (class D, 65.2%). Despite that not all accessions of the other groups were tested with this method, all the tested SNK600 accessions were found in the same class (A), being thereby classified as resistant (Table 4). The accession SNK616 had the lowest percentage of successful infections (DS = 4.3), whereas 13 accessions (including SNK10 usually known as the most susceptible SNK accession) recorded an infection rate above 90%.

Field incidence (FI) *of PPR (% of rotten pods)*

Generally, only the limited number of accessions that are

Table 2. Distribution of the accessions in PPR resistance classes based on their average disease scores in leaf disc test (LDT).

Accession group	Mean score	Resistance class	Number of accessions	Amplitude of variation	Ranking of corresponding accessions (*)
SNK	3.27	A	2	1.6-1.7	SNK 478; 111
		B	13	2.2-2.9	SNK 15; 413; 7; 277; 332; 213; 485; 37; 344; 12; 16; 269; 460
		C	33	3.0-3.9	SNK 461; 13; 32; 505; 31; 223; 422; 504; 33; 46; 404; 459; 467; 30; 203; 377; 488; 181; 417; 29; 48; 109; 64; 343; 415; 479; 414; 450; 506; 17; 480; 392; 65
		D	5	4.1-4.5	SNK 376; 267; 52; 416; 10
SNK600-Tr (SNK*ICS)	3.30	A	-	-	-
		B	2	2.2-2.5	SNK 605; 606
		C	8	3.0-3.9	SNK 609; 610; 600; 602; 607; 613; 603; 608
		D	1	4.29	SNK 601
SNK600-TrxUA (UPA*ICS)	2.48	A	0	-	-
		B	7	2.0-2.8	SNK 618; 616; 623; 619; 624; 615; 620
		C	0	-	-
		D	0	-	-
SNK600-TrxUA (UPA*SNK)	3.08	A	1	1.3	SNK 630
		B	2	2.7-2.9	SNK 626; 632
		C	4	3.0-3.9	SNK 634 ; 633; 628; 631; 627
		D	1	-	-

*Accessions are classified in ascending order (from the most to the least resistant) in each class.

planted in the field allowed us to observe the PPR incidence. The values of infection rate (percentage of rotten pods) varies from 15.74 (UPA*ICS) to 33.59 (SNK) (Table 5). No accessions were found in the susceptible class of the SNK*ICS group, and in the moderately susceptible and susceptible classes in the UPA*ICS and UPA*SNK groups. Very low percentages (<10%) of rotten pods were observed in four accessions: SNK620 (4.6%); SNK416 (7%); SNK608 (8.3%); and SNK619 (8.8%). The accession SNK10 was the most susceptible of the study with 87.5% of their pods rotten. About 90% of the UPA*ICS accessions had less than 25% (class A) compared to the groups SNK (20%); SNK*ICS (36.4%) and UPA*SNK (50%).

Analysis of PPR resistance for overall testing methods

After ranking the different groups based on their PPR resistance values in different testing methods (DPT, LDT, %SI, FI) (Table 6), the different ranks obtained were correlated (Table 7). All the correlation coefficients (*r*-values) were positive but variable (0.37-0.87) and significant (0.01-0.06). The best rank correlation was registered between %SI and FI (% of rotten pods) with an *r*=0.87 (p=0.01). The different laboratory screening methods were also positively correlated: *r*=0.60 (p=0.03) between DPT and LDT; *r*=0.61 (p=0.03) between DPT and %SI; and *r*=0.45 (p=0.05) between LDT and %SI. Correlation between FI and DPT recorded the lowest *r*-value (0.37, p=0.06).

DISCUSSION

The data obtained from the different methods of assessment clearly show a higher level of resistance of the SNK600 genetic group to PPR (accessions labeled from SNK 600 to 641), which confirms the efficiency of the selection of these genotypes in the 1970s. The data also clearly indicate that among these SNK600 accessions, genotypes issued from crosses with one Upper amazon Forastero parent show a higher level of tolerance than the ones issued from crosses between two Trinitario parents. Anyway, even among the SNK genetic group (accessions labeled from SNK 1 to 599), some clones showed a promising level of resistance to PPR, such as SNK 15, 27, 37, 64, 413, 417, 485 and 490, and are or will be included in the local breeding program.

Table 3. Distribution of the accessions in PPR resistance classes based on their average disease scores in detached pod test (DPT).

Accession group	Resistance class	Number of accessions	Amplitude of variation	Ranking of corresponding accessions*
SNK	A	2	2.0	SNK 27; 417
	B	6	2.6-4.0	SNK 15; 267; 64; 450; 461; 490
	C	31	4.2-6.0	SNK 67;478; 479; 52; 485; 500; 460; 353; 37; 17; 181; 322; 361; 416; 443; 332; 377; 452; 269; 505; 501; 343; 480; 30; 213; 422; 7; 12; 29; 33; 46; 496
	D	26	6.2-8.0	SNK 344; 413; 506; 13; 376; 467; 476; 469; 31; 111; 16; 348; 141; 404; 444; 456; 459; 488; 109; 32; 203; 35; 48; 10; 223; 415
SNK600-Tr (SNK*ICS)	A	1	2.0	SNK 604
	B	1	2.25	SNK 605
	C	8	4.1-6.0	SNK 603; 611; 613; 608; 602; 600; 609; 610
	D	2	6.75-7.0	SNK 607; 601
SNK600-TrxUA (UPA*ICS)	A	0	-	-
	B	4	3.5-4.0	SNK 616; 623; 614; 619
	C	3	5.0-5.9	SNK 620; 622; 615; 624
	D	1	-	-
SNK600-TrxUA (UPA*SNK)	A	0	-	-
	B	1	4.0	SNK 626
	C	3	4.8-5.7	SNK 629; 632; 628
	D	3	6.1-6.7	SNK 633; 627; 625

*Accessions are classified in ascending order (from the most to the least resistant) in each class.

The results of the study have shown that there is a strong genetic correlation between the level of PPR resistance and the group of accessions evaluated. The group SNK basically composed of accessions from local Trinitario origin was less resistant compared to the accessions which are hybrids between local SNK and introduced Trinitario or Upper Amazon Forastero (UAF). Low average resistance of pure local Trinitario genotypes compared to the introduced ones such as ICS was already detected by Efombagn et al. (2004) during field tests. Hybrid accessions with UAF genes were averagely more resistant than pure local and introduced Trinitario ones, and averagely more susceptible than the pure UAF accessions. UAF are known to harbor good sources of resistance to PPR (Wood and Lass, 1985) and different breeding strategies aiming at improving PPR resistance in cacao genetic banks have included a great proportion of UAF genotypes. Outstanding UAF derived progenies have always shown low susceptibility to PPR (Zhang 2011). In Cameroon a particular type of SNK accessions (so-called SNK600) was developed after hybridization with UAF (as defined in Table 1). The recent use of some of these accessions as parents of hybrid progenies in field trials already shows good levels of resistance.

Trinitario genotypes are known to perform well regarding agronomic traits such yield components. A great part of Trinitario accessions has good bean size. As consequence for breeding strategy in Cameroon, cacao cultivars deriving from hybrids between UAF and Trinitario were developed and released through seed gardens (Efombagn et al., 2009). However, PPR incidence in farmers' field remains high. This suggests that new resistant progenitors should be detected within the available germplasm in addition to the genotypes recently introduced through international cacao quarantines. The present study has contributed to confirm that some locally-selected accessions may be used as progenitors to developed new cacao hybrid or clonal cultivars with good level of resistance to PPR. Some SNK accessions which were poorly used in the past in varietal and seed production (such as SNK 413 and 490) should be intensively used in variety trials prior to their selection as progenitors in the future seed gardens.

As consequence in the breeding process, cacao genetic bank deriving from farmers' germplasm could enhance the level of genetic diversity as well the sources of resistance to PPR. Field surveys carried out by Pokou

Table 4. Distribution of the accessions in PPR resistance classes based on their average infection rates during the counting of successful infections (%SI) on attached pods.

Accession group	Mean score	Resistance class	Number of accessions	Amplitude of variation	Ranking of corresponding accessions
SNK	77.53	A	1	10.3	SNK 490
		B	2	36.0-45.9	SNK 413; 64
		C	13	55.0-74.0	SNK 267; 416; 32; 180; 213; 223; 461; 30; 67; 16; 15; 181; 450
		D	30	76.5-100	SNK 476; 269; 452; 37; 456; 48; 46; 12; 376; 478; 111; 7; 422; 344; 460; 332; 488; 52; 27; 10; 459; 13; 109; 343; 203; 277; 467; 33; 348; 415
SNK600-Tr (SNK*ICS)	14.72	A	4	6.3-19.2	SNK 600; 610; 611; 601
		B	0	-	-
		C	0	-	-
		D	0	-	-
SNK600-TrxUA (UPA*ICS)	11.6	A	4	4.3-16.7	SNK 616; 614; 618; 615
		B	0	-	-
		C	0	-	-
		D	0	-	-
SNK600-TrxUA (UPA*SNK)	12.66	A	7	4.5-25.0	SNK 625; 633; 629; 626; 628; 631; 627
		B	0	-	-
		C	0	-	-
		D	0	-	-

*Accessions are classified in ascending order (from the most to the least resistant) in each class.

et al. (2008) in Côte d'Ivoire, Opoku et al. (2011) in Ghana, Aikpokpodion et al. (2010) in Nigeria and Efombagn et al. (2007) in Cameroon have enable the detection of great sources of resistance against *Phytophthora* sp., causal agent of PPR. The exploitation of the local cacao genetic bank such as SNK accessions is rather supported by the fact the introduction of cacao elite clones through appropriate international quarantines become more and more difficult, due to the costs linked to management of that quarantine facilities. In addition, direct exchanges of cacao genetic material may harbor serious risks of disease transmission, given the fact all the five major diseases known in cacao cultivation worldwide are not distributed in all producing countries. The direct use of the best locally-selected accessions as released clonal variety might speed up the breeding achievements of this genetic material, regarding the constant increase in demand for high performing cacao cultivars in the country. The best PPR-resistant SNK accessions could be immediately distributed to the cacao farmers through budwood gardens or available cacao genetic banks, which therefore could shorten the time needed to produce planting material. However, PPR is not the only constraint of cacao production in Cameroon as well as in the others producing countries. In the selection process of PPR-resistant clonal material, emphasis should also be placed on the local selections with good yield performance (bean size, pods index, number of pods per tree).

Ranking of accessions groups were correlated to confirm the stability of the resistance patterns independently of the testing methods used. All the correlations were found positive and significant, confirming the reliability among laboratory and field screening approaches as identified earlier by Tahi et al. (2000). Other earlier studies including some SNK accessions have shown good correlation between leaf discs tests and attached pod resistance tests and among between leaf disc tests, detached pod tests and field observations of PPR incidence (Efombagn et al., 2011). The use of additional SNK accessions as potential progenitors raises the need to combine the results obtained with leaves and pods using screening tests or field observations. Correlations between pod testing methods (DPT and %SI) were positive but lower compared to the other r-values of the study. This due to the fact that the method of counting the number of successful infections developed in seventies (Blaha and Lotode, 1976), were improved by Iwaro et al. (2000) by describing the levels of the PPR evolution on pods

Table 5. Distribution of the accessions in PPR resistance classes based on their average infection rates in field observations (FI).

Accession group	Mean score	Resistance class	Accessions number (%)	Amplitude of variation	Ranking of corresponding accessions
SNK	33.59	A	2 (20.0)	7.0-11.6	SNK 37; 64; 416
		B	3 (30.0)	23.4-38.9	SNK 13; 30; 413
		C	3 (30.0)	41.0-47.6	SNK 109; 16
		D	2 (20.0)	74.6-87.5	SNK 48; 10
SNK600-Tr (SNK*ICS)	27.02	A	4 (36.4)	8.3-18.0	SNK 600; 602; 607; 608
		B	4 (36.4)	20.0-32.6	SNK 610; 609; 613; 611
		C	3 (27.2)	40.5-50.3	SNK 604; 605; 603
		D	0	-	-
SNK600-TrxUA (UPA*ICS)	15.74	A	9 (90.0)	4.6-23.6	SNK 620; 619; 614; 622; 615; 621; 624; 623; 616
		B	1 (10.0)	27.5	SNK 618
		C	0	-	-
		D	0	-	-
SNK600-TrxUA (UPA*SNK)	17.04	A	3 (50.0)	4.7-14.4	SNK 625; 633; 627
		B	3 (50.0)	23.2-2701	SNK 632; 628; 630
		C	0	-	-
		D	0	-	-

*Accessions are classified in ascending order (from the most to the least resistant) in each class.

Table 6. Ranking of genetic and accessions groups following their average disease scores or infection rates in all the four testing methods.

Genetic group	Accession group	Leaf disc test (LDT)	Detached pod test (DPT)	Infected pod (%SI)	Incidence of PPR in the field (FI)
SNK	-	3.27[1a3] (53)[2]	5.60b (66)	77.53a (46)	37.58[a] (10)
SNK600	All	2.99[b] (26)	4.96 [b](27)	13.8b (15)	20.62[b] (27)
	SNK*ICS	3.30[a] (11)	4.86[a] (12)	14.72b (4)	27.02[ab] (11)
	UPA*SNK	3.07[a] (7)	5.56[a] (7)	14.47b (7)	15.74[b] (10)
	UPA*ICS	2.48[b](8)	4.60[a] (8)	11.60b (4)	17.04[b] (6)

[1],PPR score [2]number of accessions; [3]different letters in the same column indicate significant differences at 5% probability among accessions groups tested with the same method (Student-Newman-Keuls test).

(symptoms) classified in eight stages in the PPR detached pod test rating scale. However, rankings of accessions were different from testing method to another. This may due to the fact that screening methods that use detached structures from the plant tend to miss any possible systemic signaling or reaction by the plant, which would be a good reason why *in vivo* (e.g pods or leaves attached to the plants) tests tend to be more reliable. Moreover, the overall interaction of the plant and the pathogen with the environment and its variations is not considered in detached-structures tests.

Besides screening methods and estimation of pod rot rates, other factors should be taken into account as PPR resistance components during the selection process of SNK genetic material. Berry and Cilas (1994) found that the length of fruiting cycle of Trinitario material such as SNK may contribute to their poor performance in the field because the fruit development and maturation stages coincide with the peak of PPR severity. Escape mechanisms developed by cacao genotypes such as upper Amazon accessions could have an influence on the level of field resistance to PPR as shown by Efombagn et al. (2004) in Cameroon in a study which included very few SNK accessions. Spatial distribution of pods on a cacao tree (Ndoumbè et al., 2002) should also been investigated in locally-selected cacao accessions regarding the high PPR pressure in cacao plantations in Cameroon.

Table 7. Rank correlations among accessions groups in different testing methods.

Groups	LDT	DPT	%SI	FI
LDT	-	0.60[1](0.03)[2]	0.61 (0.03)	0.37(0.06)
DPT		-	0.45 (0.05)	0.65[1] (0.03)
%SI			-	0.87[1] (0.01)
FI				-

[1]Correlation coefficient, [2]Probability.

The composition of groups of accessions tested with different screening methods do not interfere on the results founds in resistance classes. During the assessment of resistance to *Ceratocystis cacaofunesta* in cacao genotypes, Sanches et al. (2008) found that the clones from the CEPEC collection in Brazil were grouped as resistant, moderately resistant and susceptible. This was also observed in our study with the SNK and SNK600 groups of accessions which displayed different levels of resistance.

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