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RESEARCH ARTICLE

BREEDING OF COCOA TREES (THEOBROMA CACAO L.) RESISTANTTO *PHYTOPHTHORA MEGAKARYA*, AGENT OF BLACK POD DISEASE IN COTE D'IVOIRE

Boguinard Sahin Honorine Guiraud[1], Gnion Mathias Tahi[1], Klotioloma Coulibaly[1], Désiré Pokou[1], Evelyne Maryse Assi[1], Firmin Goure Bi[1], Mathurin Yves Atchii[1], Sandrine Okayo Minakou[1], Philippe Lachenaud[1] and Irié Arsène Zoro[2]

1. Centre National de Recherche Agronomique (CNRA), Programme Cacao, Opération Amélioration génétique, BP 808 Divo, Côte d'Ivoire.
2. UniversitéNanguiAbrogouad'Abidjan (UNA), UFR des Sciences de la Nature, Unité de Phytotechnie et Amélioration génétique, 02 BP 801 Abidjan 02, Côte d'Ivoire.

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Abstract

Backgrownd : Black pod disease is the cause of significant production losses of cocoa trees. This work aims to select tolerant and resistant genotypes to *Phytoththoramegakarya* within the main collection of cocoa trees of the National Center for Agronomic Research.

Methods : The artificial inoculation test on leaf discs, was used in this study to assess the susceptibility of 52 clones of high-producing cocoa trees resistant in the field to black podcausing by *P. megakarya*.

Results : Three groups of susceptibility to *P. megakarya* were demonstrated according to the reference controls. The first group is composed of two clones (IFC 1035 and CC 39) qualified as susceptible to black pod. These genotypes have respective sensitivity scores (NS) of 3 and 3.06 which are lower than those of the sensitive control NA32 (NS = 3.31). The second group is composed of 43 clones qualified as moderately resistant with sensitivity scores higher than 2.59 (PA150, moderately resistant control) and lower than 3.31 (NA32). The third group is composed of four clones qualified on the one hand as resistant (IFC 1041 and IFC 1027) with sensitivity scores higher than 1.73 (SCA6) and lower than 2.59 (PA 150) and on the other of very resistant to *P. megakarya* (NS> 1.73), with sensitivity scores greater than 1.73

Conclusion : These genotypes resistant to *P. megakarya* thus selected could constitute parents to be included in a variety improvement program with a view to the selection of plant material resistant to black pod disease.

Introduction:-

The cocoa tree [*Theobroma cacao L.*(Malvaceae)] is a perennial, tropical and endemic plant of South America (Cheesman, 1944). It is a highly prized crop around the world, mainly for its beans used for making chocolate, cosmetics, pharmaceuticals and other cocoa derivatives. The cocoa sector in Côte d'Ivoire contributes 15% of Gross Domestic Product (GDP) and 44% of export earnings (ICCO, 2014). However, Ivorian cocoa farming is

Corresponding Author:- Boguinard Sahin Honorine Guiraud
Address:- Centre National de Recherche Agronomique (CNRA), Programme Cacao, Opération Amélioration génétique, BP 808 Divo, Côte d'Ivoire.

increasingly facing many production constraints. These include the low level of use of improved plant material (Koua et al., 2018), the aging of the orchard (Assiri et al., 2009; 2016; Koua et al., 2018) and the high pressure parasitic. The latter is caused by pests such as mirids [SahlbergellasingularisHagl. (Mirideae)] (Kouamé et al., 2014) and diseases such as black pod rot, the main agent of which in Côte d'Ivoire is Phytophthorapalmivora but the most damaging agent in the field is *Phytophthoramegakarya*. In Côte d'Ivoire, black pods disease contributes around 10 to 25% of production losses. However, it can cause up to 60% losses in some regions when agro-ecological conditions are favorable for the development of the pathogen (Coulibaly, 2014).

The disease begins on the pods with the appearance of a brownish-colored spot that spreads quickly and can gradually cover the entire surface of the pod. In humid weather, the spots become covered with a whitish mycelial felting. Examination of the mycelial felting covering the diseased pod reveals the presence of numerous conidia which are the asexual reproduction organs of the fungus (Coulibaly, 2014). Conidia release ciliated zoospores which, dispersed by water, wind or insects, can contaminate new fruits. The germination of zoospores requires the presence of water so that contamination often begins at the apex of the fruit which, due to its shape, retains water in a hanging droplet in which the zoospores can move, germinate and infect the fruit (Braudeau, 1969). Infection begins with zoospores (germs) entering the stomata or through the epidermis. Germination of zoospores can also occur in water retained on fruit at the stalk attachment or between two adjacent pods.

One of the most using ways to control black pod disease is the use of phytosanitary products. However, this method is expensive and dangerous for the environment. To remedy this state of affairs, research is increasingly turning to genetic control to select cocoa trees resistant to brown rot (Nyassé et al., 1995; Tahi et al., 2000). The evaluation of the resistance of cocoa trees to pod rot by the leaf disc test represents an efficient, rapid and reliable technic tested by several authors (Nyassé et al., 1995; Tahi et al., 2000) for selecting cocoa trees resistant to this disease. Indeed, a positive and significant correlation has been demonstrated between the classification of genotypes by evaluation via test on cocoa leaves in the laboratory and the rate of brown rot of pods of these same genotypes observed in the field (Tahi et al., 2003). Therefore, the artificial inoculation test makes it possible to quickly assess the level of sensitivity of cocoa trees to black pod, which makes it possible to shorten the selection cycles of cocoa trees resistant to Phytophthorasp (Charbierski, 2000). Furthermore, the use of leaf discs instead of whole leaves is justified by the results obtained by Tahi et al. (2000). Indeed, these authors showed a non-significant interaction between clones and organ size. These results thus indicate that the behavior of the plant material on leaf discs does not vary significantly compared to their behavior on whole leaves. Thus, leaf discs, less bulky and easily allowing the use of a high number of clones per tank were used for the evaluation.

In the context of this study, the resistance of 52 clones to *P.megakarya*, an agent of brown pod rot was measured by artificial inoculations with a calibrated suspension of zoospores on leaf discs (Nyassé et al., 1995) following the protocol proposed by Tahi et al. (2000).

Plant material

The plant material is composed of 52 potentially high-producing clones from the CNRA collection including three clones of variable sensitivity to brown pod rot, used as reference controls. It isis NA 32, susceptible to disease; PA 150, moderately resistant to disease and SCA 6, resistant to disease (Tahi, 2003). The list of plant material is presented in **Table 1**.

Fungal material

The fungal material used for this study is a strain of *P. megakarya*, isolated from a pod naturally infected with brown rot. The strain was subcultured, maintained and stored on an artificial medium based on pea agar (**Figure 1**). The strain was cloned by mono-zoospore subculturing and its aggressiveness was re-tested on healthy pods.

Methods:-

Experimental design

The test wasrealisedusing a complete random block with 4 sub-blocks. Three repetitions of the test were carried out in order to have a solid database for statistical analyzes. Fifty-two cocoa clones (at the rate of 10 leaf discs per clone) were evaluated per tank. Forty leaf discs per clone were thus inoculated for all four tanks in a series. Thus, 2080 leaf discs for all 52 clones were inoculated per repeat.

Preparation of inoculum of strains of *Phytophthoramegakarya*

The inoculum usingis a suspension of zoospores of *P.megakarya*. The zoospore suspension was prepared from a strain grown on pea agar medium. The culture was incubated in the dark for six days at 26 °C. She was subsequently subjected to a 12-hour photoperiod for at least two days to induce sporocyst formation. Germination of the sporocysts was induced by heat shock by placing the culture for 15 min at 4 °C and then flooding it with 40 mL of sterile distilled water at room temperature. The suspension thus obtained was quantified using an optical microscope (Malassez cell) and the concentration was brought back to 3.105 zoospores per milliliter.

Collect of leaf samples

Two healthy, semi-august leaves located on the lower strata of the trees were taken very early in the morning (around 6.30 am) from each of the 52 clones (including the 3 controls). They were labeled, bagged and stored in a cooler containing foam soaked in distilled water. The plant material was then quickly transported to the laboratory to start the inoculations according to the protocol of Tahi et al. (2000).

Leaf inoculation

Healthy sampled leaves were preconditioned overnight to make the leaf blade more receptive. This step consisted of placing the underside of the sheet in a tray against a foam soaked in distilled water. After preconditioning, 40 leafs discs 15 mm in diameter per clone were cut from the leaf blades using a cookie cutter. The leaf discs were placed in the trays and then inoculated on the underside of the blade, by depositing 10 µL of zoospores suspension calibrated at 3.10^5 zoospores / mL using a micropipette (**Figure 2**). Subsequently, the trays containing the inoculated leaves were sealed with black plastic wrap and incubated at 26 °C in the dark for seven (7) days.

Data collect

Seven days after incubation, data were collected on each leaf disc according to the scale proposed by Blaha (1974) and updated by Nyassé et al. (1995). Data collection consisted of observing each inoculated leaf disc and assigning it a sensitivity score according to the scale of Nyassé et al. (1995) presented in **Table 2**.

Statistical analyzes of data

P. megakarya leaf sensitivity scores were analyzed with SAS 9.4 software. The statistical analysis consisted of a comparative study between the clones in order to highlight any differences between them. For this purpose, analysis of variance (ANOVA) was previously used. Any significant ANOVA ($P<0.05$) is followed by the test for the smallest significant difference (ppds) in order to classify the different clones for the characteristic considered. Furthermore, a Dunnett test was used in order to define the grouping of the clones according to each of the three controls (resistant, moderately resistant and sensitive). Indeed, the post-hoc test (or multiple comparison test) was used to determine the significant differences between the mean sensitivity scores of the clones and those of each of the three controls.

Results:-

Table 3 shows the results of Dunett's test showing the significance of the difference between the sensitive control NA 32 and the 49 clones analyzed. Analysis of the table indicates that all the clones analyzed, with the exception of IFC 1035 (sensitivity score = 3) and CC 39 (sensitivity score = 3.06) presented sensitivity scores significantly lower than the sensitive control NA 32 (sensitivity score = 3.31).

Table 4 shows the results of Dunett's test showing the significance of difference between the moderately resistant control PA 150 and the 47 clones analyzed. Indeed, the two clones identified as sensitive were removed from the database before performing the statistical analyzes. The results indicate that four clones (IFC 1041; IFC 1027; GU 346 / R; GU 322 / B) exhibited respective sensitivity scores of 1.54; 1.63; 1.47; 1.36; significantly lower than the moderately resistant control PA 150 (NS = 2.59). These clones could be qualified as resistant or very resistant to brown pod rot. The remaining 43 clones [2.59 (PA150) <NS <3.31 (SCA6)] showed higher sensitivity scores than the moderately resistant control (PA150) (NS = 2.59) and lower than the resistant control (SCA6). These genotypes could therefore be qualified as moderately resistant

The results of Dunett's test showing the significance of the difference between the resistant control SCA 6 and the four clones analyzed are presented in **Table 5**. Indeed, the 43 clones identified as being moderately resistant were removed from the database before to perform statistical analyzes. The table Indicates that the two clones (IFC 1041 and IFC 1027) presented respective sensitivity scores of 1.54 and 1.63, higher than that of the resistant control SCA

6 and lower than that of the moderately resistant control [1.73 (SCA6) <NS <2.59 (PA 150)]. These clones could be described as resistant to brown pod rot. Finally, the two remaining clones (GU 346 / R; GU 322 / B) presented respective sensitivity scores of 1.47 and 1.36, lower than those of the resistant control SCA 6 (NS = 1.73). These clones could be described as very resistant to brown rot.

Table 6 presents the mean values of the susceptibility scores to *P. megakarya* of potentially high-producing clones. Table indicates that the leaf sensitivity scores of the clones to this pathogen are between 1.36 (GU 322 B) and 3.31 (NA 32) with an average of 2.38 ± 0.74 and a coefficient variation of 14.18. The results indicates a very highly significant difference (P<0.0001) between the 52 clones analyzed for *P. megakarya* infection scores. The results of this table show the structuring of the genotypes according to the 3 different groups of sensitivities.

Discussion:-

This study consisted of the evaluation of the resistance by leaf disc test to *P. megakarya* of 52 clones potentially high producers and resistant to brown pod rot in the field. This work was undertaken to better appreciate the tolerance to brown rot of the clones evaluated.

The results of this study showed a very highly significant difference (P<0.001) between the 50 clones analyzed for the scores of susceptibility to *P. megakarya*. Three levels of sensitivity make it possible to structure the analyzed clones. Indeed, the results made it possible to highlight three groups of sensitivity according to the reference controls. The first group is composed of two clones (IFC 1035 and CC 39) qualified as susceptible to brown rot. The second group is made up of 43 clones qualified as moderately resistant. The third group is composed of four clones qualified as resistant (IFC 1041 and IFC 1027) and very resistant (GU 346 / R; GU 322/B) to the pathogen. These results confirm the horizontal nature of the resistance to brown pod rot that would characterize cocoa trees, as indicated by Tahi (2003). In addition, the clones qualified as very resistant belong to the "Guiana" genetic group (GU 346 / R; GU 322 / B). These results are in agreement with those of Paulin et al. (2008) whose work focused on identifying new sources of resistance to *P. megakarya* in cocoa trees. These authors identified seven (07) and 29 new clones of the Guiana genetic group, respectively very resistant and resistant to *P. megakarya*. The results of these authors also showed that the clones of this genetic group would constitute sources of resistance to *P. megakarya*. Their study thus confirmed the good level of resistance of the clones of the "Guiana" group to *P. megakarya* and the important role that the clones of this genetic group could play in a breeding program. This program would aim to control the pathogen and select clones tolerant to brown pod rot (Dzahini-Obiatey& Fox, 2010). Moreover, based on the existence of a positive and significant correlation highlighted by the work of Tahi et al. (2000), clones GU 346 / R and GU 322 / B, which presented lower sensitivity scores than the resistant control SCA6, will be characterized by a high resistance toBlack pod disease.

Conclusion:-

This work consisted of an evaluation of resistance to *P. megakarya* of 52 cocoa clones notable for production and resistance in the field to brown pod rot.

The results revealed three groups of genetic diversity, within the population studied, according to their susceptibility to *P. megakarya*. The first group is composed of two clones (IFC 1035 and CC 39) sensitive to *P. megakarya*. The second group consists of 43 clones moderately resistant to brown rot. The third group is composed of four clones qualified as resistant (IFC 1041 and IFC 1027) and very resistant (GU 346 / R; GU 322 / B) to the pathogen. In addition, the group qualified as very resistant to brown rot was marked by a strong contribution from clones of the Guiana genetic group.

These results constitute an important source of information for the breeder and an indicator in the choice of clones to be proposed for variety release. In addition, these high-performance clones constitute broodstock to be integrated into a breeding program for plant material resistant to brown pod rot.

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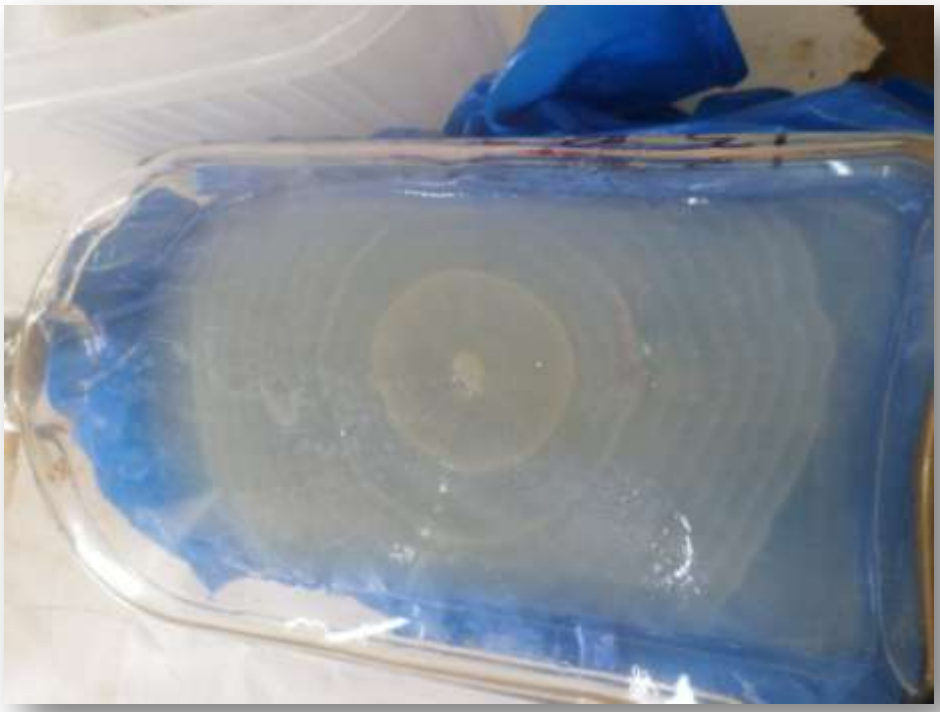

Figure 1:- *P. megakarya* strain cultivated on agar medium, used for inoculation by test on leaves of 52 clones of potentially high-producing cocoa trees and resistant to brown pod rot under natural infestation conditions.

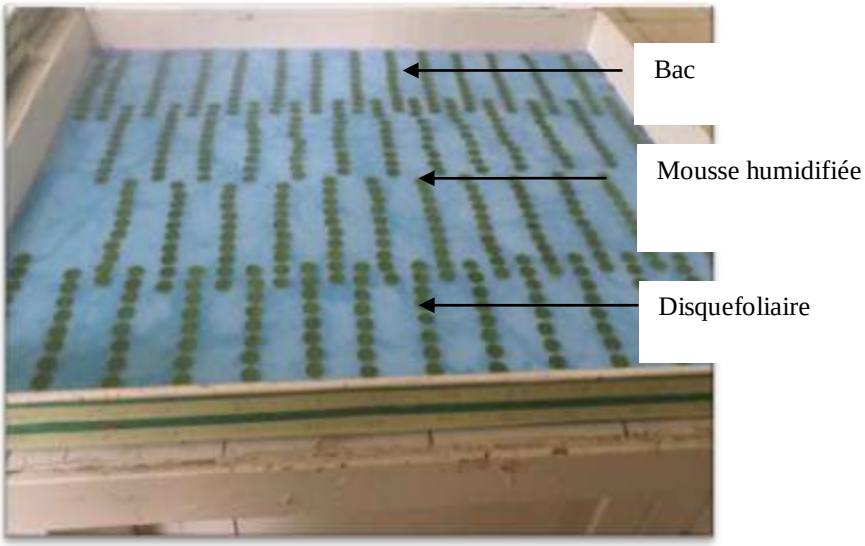

Figure 2:- Arrangement of leaf discs in a bin.

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Table 1:- List and characteristics of 52 cocoa clones from the CNRA collection A 21 used for the evaluation for P. megakarya.

Number	Clones	Type	Genealogy

1	**IFC 1058**	Cloned hybrid	UPA 418 X IFC 1
2	**IFC 1028**	Cloned hybrid	GU 165 X UPA 605
3	**IFC 1027**	Cloned hybrid	T 3 X UPA 603
4	**IFC 1041**	Cloned hybrid	UPA 603 X IFC 409
5	**IFC 1026**	Cloned hybrid	T 3 X UPA 603
6	**GU 343/F**	Guiana	-
7	**CC 39**	Cloned hybrid	Matina X Unknown
8	**DLM 2**	Cloned hybrid	M 2 X Unknown
9	**IFC 1035**	Cloned hybrid	UPA 206 X IFC 1
10	**IFC 1201**	Cloned hybrid	PA 150 X IFC 5
11	**IFC 698**	Cloned hybrid	T 60/974 X Unknown
12	**IFC 16**	Local Trinitario	T16
13	**SF 151**	Local Trintario	-
14	**GU 322/B**	Guiana	-
15	**IFC 1038**	Cloned hybrid	UPA 603 X IFC 5
16	**IFC 1025**	Cloned hybrid	T 38 X UPA 402
17	**IFC 1210**	Cloned hybrid	H 985 X IFC 5
18	**IFC 1209**	Cloned hybrid	DLH 809
19	**SF 143**	Local Trinitario	-
20	**IFC 1060**	Cloned hybrid	UPA 418 X IFC 1
21	**GTW**	Catongo	-
22	**IFC 1208**	Cloned hybrid	H 692 X IFC 5
23	**R 15**	Trinitario	-
24	**IFC 1059**	Cloned hybrid	UPA 418 X IFC 1
25	**IFC 706**	Cloned hybrid	-
26	**IFC 1062**	Cloned hybrid	UPA 606 X IFC 405
27	**GU 343/K**	Guiana	
28	**IFC 1048**	Cloned hybrid	UPA 614 X IFC 1
29	**ACU 85**	Trinitario	-
30	**DL E31**	Cloned hybrid	E 31 X Unknown
31	**IFC 705**	Cloned hybrid	T 60/974 X Unknown
32	**CF 62**	Cloned hybrid	Tinitario X Amelonado
33	**SF 153**	Amelonado	-
34	**SPEC 160-9**	Cloned hybrid	-
35	**IFC 682**	Cloned hybrid	T 60/974 X Unknown
36	**GU 342/G**	Guiana	-
37	**IFC 1029**	Cloned hybrid	UPA 113 X IFC5
38	**SF 73**	Trinitario	-
39	**UPA 705**	Cloned hybrid	T 85/78 X T 87/1309
40	**IFC 6**	Trinitario	

Table 1 (following). List and characteristics of 52 cocoa clones from CNRA collection used for *P. megakarya* assessment

Number	Clones	Type	Genealogy
41	**SF 152**	Local Trinitario	-
42	**IFC 679**	Cloned hybrid	T 60/974 X Unknown
43	**GU 346/R**	Clone	-
44	**IFC 1037**	Cloned hybrid	UPA 517 X IFC 5
45	**IFC 1202**	Cloned hybrid	PA 7 X IFC 15
46	**IFC 1044**	Cloned hybrid	UPA 409 X IFC 412
47	**IFC 683**	Cloned hybrid	PM 86
48	**P4/9 (J114/5)**	Clone	-
49	**IFC 1039**	UPA 603 X IFC 5	UPA 603 X IFC 5
50	**NA 32**	Forasteroupper amazon	Control

51	**PA 150**	Forastero upper amazon	Control
52	**SCA 6**	Forastero upper amazon	Control

Table 2:- Notes of infections and types of symptoms that can be seen on the discsleaves after inoculation with *Phytoththora spp*.

Infection notes	Type of symptoms	Meaning
0	No symptom	Very resistant
1	Single point of penetration (tiny necrotic point)	resistant
2	Network penetration points (several necrotic points)	Moderately resistant
3	Networked tasks (joining task	Moderately resistant
4	Marble spots (large, uniform spots)	sensitive
5	True spots (very large necrotic spots, sometimes exceeding the limits of the inoculation drop)	Very sensitive

Table 31:- Probability (from Dunnett's test) showing the significance of the differencebetween the clones and the sensitive control NA 32.

Number	Clones	Sensitivity scores	Probability
1	IFC 1210	1.96 ± 0.27^{hijklmno}	0.000023
2	IFC 1208	2.24 ± 0.43^{efghijkl}	0.000023
3	IFC 1209	$2.51 \pm 0.46^{bcdefghij}$	0.000023
4	IFC 1202	1.91 ± 0.23^{jklmno}	0.000023
5	IFC 1201	2.7 ± 0.21^{bcdef}	0.000024
6	IFC 1062	2.58 ± 0.16^{bcdefgh}	0.000023
7	IFC 1060	2.59 ± 0.42^{bcdefg}	0.000023
8	IFC 1059	2.6 ± 0.28^{bcdefg}	0.000023
9	IFC 1058	2.58 ± 0.39^{bcdefgh}	0.000023
10	IFC 1048	2.78 ± 0.27^{bcde}	0.000133
11	IFC 1044	2.7 ± 0.57^{bcdef}	0.000024
12	IFC 1041	1.54 ± 0.2^{nop}	0.000023
13	IFC 1039	2.75 ± 0.59^{bcde}	0.000103
14	IFC 1038	2.58 ± 0.44^{bcdefgh}	0.000023
15	IFC 1037	$2.18 \pm 0.47^{efghijklm}$	0.000023
16	IFC 1035	3 ± 0.3^{abc}	**0.146230**
17	GTW	$2.51 \pm 0.44^{bcdefghij}$	0.000023
18	SPEC 1609	2.68 ± 0.35^{bcdef}	0.000023
19	IFC 1025	2.23 ± 0.14^{efghijkl}	0.000023
20	IFC 1026	1.99 ± 0.53^{hijklmno}	0.000023
21	IFC 1027	1.63 ± 0.27^{mnop}	0.000023
22	IFC 1028	2.08 ± 0.26^{fghijklm}	0.000023
23	GU 342/G	1.64 ± 0.32^{mnop}	0.000023
24	GU 343/K	1.85 ± 0.4^{klmnop}	0.000023
25	GU 346/R	1.47 ± 0.21^{op}	0.000023

Any value of $P<0.05$ indicates that the mean of the clone is lower than that of the control (M <Control)

Table 3 (following):- Probability (from Dunnett's test) showing the significance of thedifference between the clones and the sensitive control NA 32.

Number	Clones	Sensitivity scores	Probability
26	GU 322/B	1.36 ± 0.13^{p}	0.000023
27	DLE 31	$2.44 \pm 0.29^{bcdefghijk}$	0.000023
28	CF 62	$2.45 \pm 0.48^{bcdefghij}$	0.000023
29	DLM 2	2.92 ± 0.3^{abcd}	0.011390
30	CC 39	3.06 ± 0.59^{ab}	**0.218843**
31	SF 161	2.67 ± 0.37^{bcdef}	0.000023

32	SF 153	2.65 ± 0.57^{bcdef}	0.000023
33	SF 152	2.4 ± 0.38^{cdefghijk}	0.000023
34	SF 143	$2.46 \pm 0.68^{bcdefghij}$	0.000023
35	SF 73	1.95 ± 0.21^{ijklmno}	0.000023
36	R 15	1.95 ± 0.21^{bcdef}	0.000023
37	ACU 85	-	0.000023
38	IFC 6	2.33 ± 0.25^{defghijk}	0.000023
39	IFC 16	2.68 ± 0.64^{bcdef}	0.000023
40	P4/9 (G114/5)	-	0.000023
41	IFC 679	2.4 ± 0.64^{cdefghijk}	0.000023
42	IFC 682	2.71 ± 0.43^{bcdef}	0.000024
43	IFC 683	2.66 ± 0.37^{bcdef}	0.000023
44	IFC 698	2.54 ± 0.4^{bcdefghi}	0.000023
45	IFC 705	$2.14 \pm 0.52^{efghijklm}$	0.000023
46	IFC 706	2.53 ± 0.41^{bcdefghi}	0.000023
47	UPA 604	2.01 ± 0.25^{hijklmno}	0.000023
48	UPA705	$2.41 \pm 0.43^{cdefghijk}$	0.000023
49	PA 4	2.71 ± 0.53^{bcdef}	0.000092
50	NA 32 (témoin)	3.31 ± 0.19^{a}	

Any value of P<0.05 indicates that the mean of the clone is lower than that of the control (M <Control)

Table 4:- Probability (from Dunnett's test) showing the significance of the differencebetween 48 clones and the moderately resistant control PA 150.

Number	**Clones**	**Sensitivity scores**	**Probability**
1	IFC 1210	1.96 ± 0.27^{hijklmno}	0.946162
2	IFC 1208	2.24 ± 0.43^{efghijkl}	0.999977
3	IFC 1209	$2.51 \pm 0.46^{bcdefghij}$	0.999977
4	IFC 1202	1.91 ± 0.23^{jklmno}	0.928709
5	IFC 1201	2.7 ± 0.21^{bcdef}	0.999977
6	IFC 1062	2.58 ± 0.16^{bcdefgh}	0.999977
7	IFC 1060	2.59 ± 0.42^{bcdefg}	0.999977
8	IFC 1059	2.6 ± 0.28^{bcdefg}	0.999977
9	IFC 1058	2.58 ± 0.39^{bcdefgh}	0.999977
10	IFC 1048	2.78 ± 0.27^{bcde}	0.999977
11	IFC 1044	2.7 ± 0.57^{bcdef}	0.999977
12	IFC 1041	1.54 ± 0.2^{nop}	0.003833
13	IFC 1039	2.75 ± 0.59^{bcde}	0.999977
14	IFC 1038	2.58 ± 0.44^{bcdefgh}	0.999977
15	IFC 1037	$2.18 \pm 0.47^{efghijklm}$	0.999976
16	GTW	$2.51 \pm 0.44^{bcdefghij}$	0.999977
17	SPEC 160	2.68 ± 0.35^{bcdef}	0.999977
18	IFC 1025	2.23 ± 0.14^{efghijkl}	0.989894
19	IFC 1026	1.99 ± 0.53^{hijklmno}	**0.041418**
20	IFC 1027	1.63 ± 0.27^{mnop}	0.999543
21	IFC 1028	2.08 ± 0.26^{fghijklm}	0.054879
22	GU 342/G	1.64 ± 0.32^{mnop}	0.775729
23	GU 343/K	1.85 ± 0.4^{klmnop}	0.000483
24	GU 346/R	1.47 ± 0.21^{op}	0.000092

Any value of *P*<0.05 indicates that the mean of the clone is lower than that of the control (M <Control)

Table 4 (following):- Probability (from Dunnett's test) showing the significance of the differencebetween 48 clones and the moderately resistant control PA 150.

Number	**Clones**	**Sensitivity scores**	**Probability**
25	GU 322/B	1.36 ± 0.13^{p}	0.999977

26	DLE 31	$2.44 \pm 0.29^{bcdefghijk}$	0.999977
27	CF 62	$2.45 \pm 0.48^{bcdefghij}$	0.999977
28	DLM 2	2.92 ± 0.3^{abcd}	0.999977
29	SF 161	2.67 ± 0.37^{bcdef}	0.999977
30	SF 153	2.65 ± 0.57^{bcdef}	0.999977
31	SF 152	2.4 ± 0.38^{cdefghijk}	0.960042
32	SF 143	$2.46 \pm 0.68^{bcdefghij}$	0.999977
33	SF 73	1.95 ± 0.21^{ijklmno}	0.999977
34	R 15	1.95 ± 0.21^{bcdef}	0.999977
35	ACU 85	-	0.999977
36	IFC 6	2.33 ± 0.25^{defghijk}	0.907287
37	IFC 16	2.68 ± 0.64^{bcdef}	0.999977
38	P4/9(j114/5)	-	0.999977
39	IFC 679	2.4 ± 0.64^{cdefghijk}	0.999977
40	IFC 682	2.71 ± 0.43^{bcdef}	0.999977
41	IFC 683	2.66 ± 0.37^{bcdef}	0.999903
42	IFC 698	2.54 ± 0.4^{bcdefghi}	0.999977
43	IFC 705	$2.14 \pm 0.52^{efghijklm}$	0.998112
44	IFC 706	2.53 ± 0.41^{bcdefghi}	0.999977
45	UPA 604	2.01 ± 0.25^{hijklmno}	0.999977
46	UPA 705	$2.41 \pm 0.43^{cdefghijk}$	
47	PA 4	2.71 ± 0.53^{bcdef}	
48	PA 150 (control)	2.59 ± 0.7^{bcdefg}	-

Any value of $P<0.05$ indicates that the mean of the clone is lower than that of the control (M <Control)

Table 5:- Probability (from Dunnett's test) showing the significance of the differencebetween the clones and the resistant control SCA 6.

Number	Clones	Sensitivity scores	Probability
1	IFC 1041	1.54 ± 0.2^{nop}	0.106011
2	IFC 1027	1.63 ± 0.27^{mnop}	0.388250
3	GU 346/R	1.47 ± 0.21^{op}	0.027326
4	GU 322/B	1.36 ± 0.13^{p}	0.000998
5	SCA 6 (control)	1.73 ± 1.18^{lmnop}	-

Any value of $P<0.05$ indicates that the mean of the clone is lower than that of the control (M <Control)

Table 6:- Average values (± standard deviation) of leaf sensitivity scores to *Phytophthoramegakarya* of 50 cocoa clones from the CNRA collection.

Number	Clones	Sensitivity scores
1	GU322 B	1.36 ± 0.13^{p}
2	GU346/R	1.47 ± 0.21^{op}
3	IFC 1041	1.54 ± 0.2^{nop}
4	IFC 1027	1.63 ± 0.27^{mnop}
5	GU 342/G	1.64 ± 0.32^{mnop}
6	SCA 6	1.73 ± 1.18^{lmnop}
7	GU 343/K	1.85 ± 0.4^{klmnop}
8	IFC 1202	1.91 ± 0.23^{jklmno}
9	SF 73	1.95 ± 0.21^{ijklmno}
10	IFC 1210	1.96 ± 0.27^{hijklmno}
11	IFC 1026	1.99 ± 0.53^{hijklmno}
12	UPA 604	2.01 ± 0.25^{hijklmno}
13	IFC 1028	2.08 ± 0.26^{fghijklm}
14	IFC 705	$2.14 \pm 0.52^{efghijklm}$
15	IFC 1037	$2.18 \pm 0.47^{efghijklm}$
16	IFC 1025	2.23 ± 0.14^{efghijkl}

Group 3: Very resistant/resistant clones

Group 2: Moderately resistant clones

17	IFC 1208	2.24 ± 0.43^{fghijklm}
18	IFC 6	2.33 ± 0.25^{defghijk}
19	IFC 679	2.4 ± 0.64^{cdefghijk}
20	SF 152	2.4 ± 0.38^{cdefghijk}
21	UPA 705	2.41 ± 0.43^{cdefghijk}
22	DLE 31	2.44 ± 0.29^{bcdefghijk}
23	CF 62	2.45 ± 0.48^{bcdefghij}
24	SF 143	2.46 ± 0.68^{bcdefghij}
25	GTW	2.51 ± 0.44^{bcdefghij}

*For each parameter, the means bearing the same letters are statistically identical to the threshold $P<0.05$

Table 6 (following):- Average values (± standard deviation) of leaf sensitivity scores to *Phytophthoramegakarya* of 50 cocoa clones from the CNRA collection.

Number	**Clones**	**Sensitivity scores**
26	IFC 1209	2.51 ± 0.46^{bcdefghij}
27	IFC 706	2.53 ± 0.41^{bcdefghi}
28	IFC 698	2.54 ± 0.4^{bcdefghi}
29	IFC 1038	2.58 ± 0.44^{bcdefgh}
30	IFC 1058	2.58 ± 0.39^{bcdefgh}
31	IFC 1062	2.58 ± 0.16^{bcdefgh}
32	IFC 1060	2.59 ± 0.42^{bcdefg}
33	PA 150	2.59 ± 0.7^{bcdefg}
34	IFC 1059	2.6 ± 0.28^{bcdefg}
35	SF 153	2.65 ± 0.57^{bcdef}
36	IFC 683	2.66 ± 0.37^{bcdef}
37	SF 161	2.67 ± 0.37^{bcdef}
38	IFC 16	2.68 ± 0.64^{bcdef}
39	R 15	2.68 ± 0.23^{bcdef}
40	SPEC 160	2.68 ± 0.35^{bcdef}
41	IFC 1044	2.7 ± 0.57^{bcdef}
42	IFC 1201	2.7 ± 0.21^{bcdef}
43	IFC 682	2.71 ± 0.43^{bcdef}
44	PA 4	2.71 ± 0.53^{bcdef}
45	IFC 1039	2.75 ± 0.59^{bcde}
46	IFC 1048	2.78 ± 0.27^{bcde}
47	DLM 2	2.92 ± 0.3^{abcd}
48	IFC 1035	3 ± 0.3^{abc}
49	CC 39	3.06 ± 0.59^{ab}
50	NA 32	3.31 ± 0.19^{a}
	DDL	**50**
	Mean	**2.38 ± 0.74**
	CV	**14.18**
	F	**12.82**
	P	**< 0.0001**

Group 2: Moderately resistant clones

Group 3: Sensitive clones

*For each parameter, the means bearing the same letters are statistically identical to the threshold $P<0.05$

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