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Original Research Article

Guaiacol Peroxidase heritability in tolerance of cocoa (*Theobroma cacao* L.) to *Phytophthora megakarya*, agent of cocoa black pod disease

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Claude Simo*[1,4], Pierre François Djocgoue [2,4] Emile Minyaka [3,4], and Ndoumou Denis Omokolo[4]

[1]Department of Plant Biology, Faculty of Science, University of Douala, PO Box 24157 Douala, Cameroon.
[2]Department of Plant Biology, Faculty of Science, University of Yaoundé I, P.O. Box 812, Yaoundé, Cameroon.
[3]Department of Biochemistry, Faculty of Science, University of Douala, PO Box 24157 Douala, Cameroon.
[4]Laboratory of Plant Physiology, Department of Biological Sciences, Higher Teacher's Training College, University of Yaoundé I, P.O. Box 47, Yaoundé, Cameroon.

Tel: (+237) 677 58 92 87/ 691 78 36 07

*Corresponding Author E-mail: simoclaude@yahoo.fr; simoclaude@univ-douala.com

Black pod disease (BPD) caused by *Phytophthora megakarya* is the main limiting factor of cocoa production in African countries. This pathogen is responsible of yield reductions of 30-80%. Developing cocoa genotypes tolerant to BPD is the way out to improve yield and cocoa sustainability. Plants exposed to biotic stresses adjust their physiology and metabolism (such as stress-related enzymes among which peroxidases). This study was focused on peroxidases activities and heritability of (POX) in two hybrid populations F13 (♀SNK13 x ♂T79/467) and F79 (♀T79/467 x ♂SNK13) subjected *P. megakarya.* The results show that more tolerants and more productive hybrid genotypes (F1307, F1314, F7902, F7928) and more tolerant genotypes (F1315, F1313, F7926, F7907) relative to the best parent in the soluble (S) and bound fractions (L) recorded a large amount of POX activity with small areas of necrotic lesion in contrast to less tolerant and productive genotypes (F1324, F1308, F7915 and F7919) which displayed lowest POX activities and largest areas of necrotic lesion. A negative and significant correlation (P <0.01) was observed between the development of necrosis and peroxidase activities. The profile of peroxidase isoforms (S) of the mesocarp of infected pods revealed the existence of a specific form (A2) after infection in tolerant genotypes T79 / 467, F7902, F7926, F1315 and F1307. This isoform is linked to tolerance. The heritability values of POX activity obtained in soluble fractions (S) in the F13 family (♀SNK13 x ♂T79 / 467) in the F79 family (♀T79 / 467 x ♂SNK13) were relatively high, they were 0.65 and 0.62 respectively. These high values show a strong additive variance in the transmission of tolerance to black pod disease. The manifestation of hybrid vigor and the heritability values of POX that have been inherited by the offspring indicate a good general aptitude for the combination of parental clones. The existing isoform (A2) in hybrid tolerant genotypes could be used to develop productive and tolerant genotypes for farmers.

Key words: *Theobroma cacao*, *Phytophthora megakarya*, black pod disease, heritability, tolerance, peroxidases.

INTRODUCTION

Cocoa, although originating from the upper Amazon basin, is cultivated in the humid tropics of the world (Motamayor et al., 2002, Yanelis et al., 2012). 80% of cocoa production produced by small-scale farmers enable these farmers to be employed in many rural communities (Curry et al., 2007, Hasna et al., 2011, Minimol et al., 2015, Ngoh Dooh et al.,

2015). Africa is currently the largest cocoa producing continent with about 73.7% of world production. Among these producing countries, five are distinguished by their active participation in cocoa cultivation, particularly Côte d'Ivoire (40.7%), Ghana (20.2%), Indonesia (7.2%), Cameroon (5.5%) and Ecuador (5%) (Anonymous, 2016, Effa et al., 2017). The cultivation of cocoa (*Theobroma cacao* L.) is a source of income for producing countries. Many people around the world depend on cocoa production and transformation related activities.

However, the cocoa cultivation is facing many difficulties among which, the aging of the plantations and diseases are responsible for cocoa low yielding. Black pod disease caused by *Phytophthora megakarya* and *Phytophthora palmivora* remain the most disastrous diseases in cocoa plantation in Africa (Shahin et al., 2017a; Shahin et al., 2017b). These pathogens have dramatic social and economic consequences in cocoa producing countries in West and Central Africa, demonstrating the extent of damage they could cause in the absence of any phytosanitary treatment (Fagbohun and Aderiye, 2017). In Cameroon, *P. megakarya* is reported as the causal agent of disease in cocoa plantation (Nyasee et al., 1995). This species can damage 50 to 80% of cocoa production or up to 100% if no phytosanitary treatment is carried out (Nyasse 1997, Ndoumbe-Nkeng et al., 2004).

The management of harmful consequences of black pod disease has become a priority for cocoa production improvement. The main sustainable strategy to minimize black pod disease incidence (on cocoa production and profitability) is genetic control. Genetic control of BPD aims to develop hybrid genotypes tolerant/resistant to BPD. This seems to be better than chemical control approach based on the use of chemical fungicides which are costly, restrictive, uneffecient and environmentally unfriendly (Ndoumbe-Nkeng 2002, Nyasse et al., 2002; Deberdt et al., 2008, Nyadamu et al. al., 2013).

The limiting factor for the implementation of genetic control strategy is the targeting of adequate parents genotypes and the efficient method for selecting tolerant/resistant hybrid genotypes with additive effect of parental genes for their use to establish cocoa plantation in order to enhance the production and profitability (Djocgoue et al., 2006, Boudjeko et al., 2007).

The interactions cocoa/*P. megakarya* were studied. Anatomical, histological and biochemical incidences related to cocoa/*P. megakarya* interactions were reported (Bailey et al., 2015).

When expose to *P. megakarya* infection, T. cacao adjust its metabolism in order to limit biotic stress incidences. The physical perception of biotic stress is observed through the development of necrosis on pods. Recent studies reported a correlation between necrosis surface on cocoa pods artificially inoculated and tolerance / resistance against *P. magakarya* Development of necrosis is stress-related response which may involve stress-related enzymes such as peroxidases.

Peroxidases are involved in a wide range of plant physiological processes, which include the cross-linking of cell wall components during cell wall formation and modification, lignification, and tuberization (Fry, 1986; Brownleader et al. al., 1995; Bernards et al., 1999; Ostergaard et al., 2000; Marjamaa et al., 2009; Huai-Fu et al., 2014; Afroz et al., 2017). They intervene in plant defense responses to parasitic attacks, such as bacteria, fungi and viruses (Delannoy et al., 2003; Diaz-Vivancos et al., 2003; Delannoy et al., 2006; Chassot et al. al., 2007; Lethonen et al., 2009; Xue et al., 2017). These enzymes are involved on the one hand in the cross-linking of the components of the cell wall, which strengthens this cell wall to hinder the invasion of pathogens and on the other hand, have the ability to generate reactive oxygen species that produce adverse circumstances for the survival of pathogens and / or to trigger downstream signaling pathways to activate additional defense mechanisms (Bindschedler et al., 2006; Torres et al., 2006; Almagro et al., 2006; Bolwell and Daudi 2009; Jin et al., 2011).

This study aims (1) to evaluate the heterosis effect of peroxidases in hybrid genotypes, (2) to estimate the heritability of this defense enzyme through quantitative analyzes and (3) to identify the isoform(s) of these peroxidases involved in the tolerance / resistance of *T. cacao* against this pathogen through the qualitative analyzes in healthy, wounded and wounded inoculated pods.

MATERIAL AND METHODS

Plant material

The plant material used in this work consists of immature cocoa pods belonging to two parental clones (SNK13, less tolerant and more productive and T79 / 467, more tolerant and less productive) and their hybrid progenies grouped within families (progeny) F13 (♀SNK13x♂T79 / 467) and F79 (♀ T79 / 467x♂SNK13) from the experimental station of the Mengang Cocoa Development Corporation (SODECAO). The fungal material is a local strain of *Phytophthora megakarya* (TA121) from the Agricultural Research Institute for Development (IRAD) of the Nkolbisson Research Station.

Inoculation of cocoa pods

Pods of 3 months-old, were harvested, washed with tap water and then disinfected with 70 °C alcohol soaked cotton and divided into three groups: the group of healthy pods (H); the group of sacrified pods inoculated with sterilised agar disk (S) and the group of scarified pods inoculated with an agar disc containing *P. megakaya* mycelia (I).

A strain of *Phytophthora megakarya* maintained by regular transfers on peas medium is used for the inoculation of pods. Inoculation is done by depositing an agar disk of 6 mm diameter, containing mycelia on the wound made using A punch. These cuts are blocked with cotton soaked in

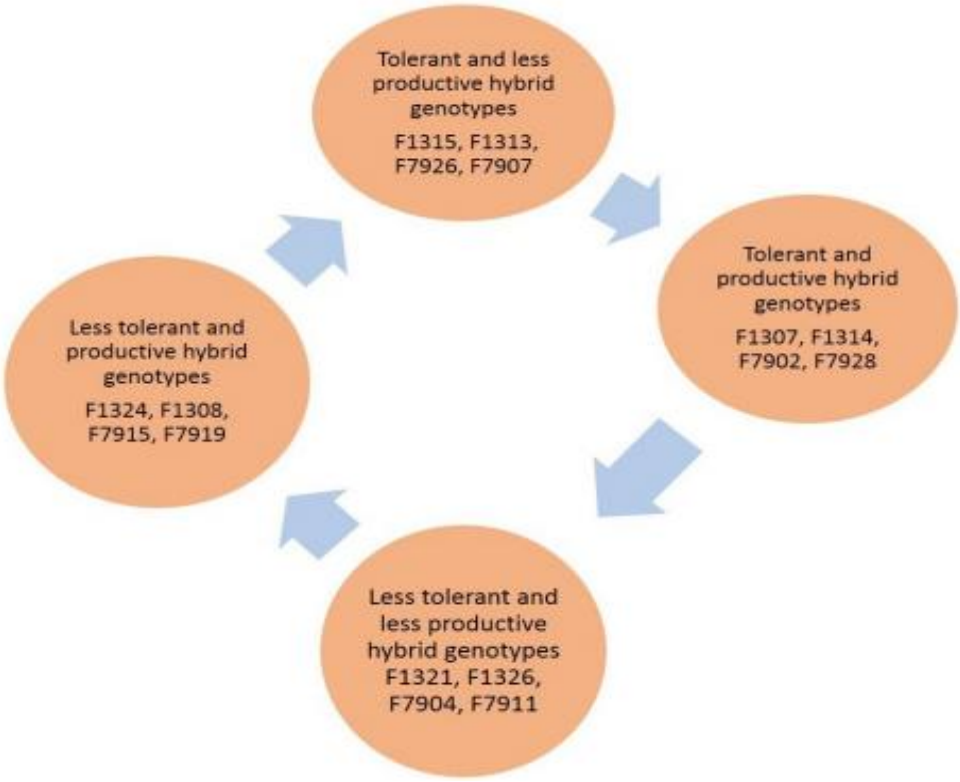

Figure 1: Different groups of hybrid genotypes for the studies of peroxidasic activities

sterile water. Inoculation of pods occurs in the culture room at 25 °C and incubation in the dark at 25 °C (Omokolo et al., 2003).

Evaluation of the development of necrosis

The development of necrosis was measured over three consecutive cocoa campaigns with three replicates for each campaign. Measurements of the surface of the necrosis are made within 3, 4, 5 and 6 days after inoculation. The diameters of the more or less circular necrotic spots are measured and the area of the necrosis is calculated from the formula of Blaha and Lotode (1976).

$S=D\times d\times \Pi /4$

S = Surface of the necrosis (cm^2)
D = Large diameter of the necrosis (cm)
d = Small diameter of the necrosis (cm)
Π = 3.14

Identification of genotypes for studies of peroxidase activities

The parental and hybrid genotypes identified for peroxidase activities studies were subdivided into four groups: (1) tolerant and productive (F1307, F1314, F7902, F7928), (2) tolerant and less productive (F1315, F1313, F7926, F7907), (3) less tolerant and productive (F1324, F1308, F7915, F7919) and (4) less tolerant and less productive (F1321, F1326, F7904, F7911) (Djocgoue et al., 2010) (Figure 1).

Peroxidases extraction

Soluble and wall-bound proteins are extracted from the modified Legrand and Dubois (1977) method. One gram of frozen plant material (made of mesocarp of *Theobroma cacao* L.) is ground in acetone 100 %. After filtration under vacuum, one g of obtained powder were ground in 3 ml of 50 mm Tris-Maleate buffer PH 7 supplemented with 0.5 M manitol (buffer A) after addition of a pinch of fine sand and polyvinylpyrrolidone (PVP). The mixture was then incubated at 4 °C for 60 min. After, the homogenate was centrifuged at 6000 g (JOUAN centrifuge) for 30 min, the supernatant (S1) collected constitutes the crude extract of soluble peroxidases.

The pellet (P1) was resuspended in the same buffer (buffer descried above) and homogenized. After 60 min of incubation at 4 °C, the mixture is centrifuged at 6000 g for 30 min, the resulting supernatant was eliminated. This operation was done twice. After, the collected pellet was resuspended and homogenized in 1.5 ml Tris-Maleate buffer (10 mM, pH 7) containing 1 M NaCl (Buffer B). The homogenate was incubated for 60 min at 4° C and then centrifuged (6000g 30 min). The resulted supernatant (S4)

Table 1. Solutions of the revelation of peroxidases

Solution1	Solution 2	Revealing Solution
-2 g of benzidine	-100 ml of 3% hydrogen	-10 ml of solution 1
-18 ml of pure acetic acid	peroxide	-6.4 ml of solution 2
-72 ml of distilled water		-304 ml of distilled water

Dark blue bands appear after 2 to 3 minutes. The results are saved as Zymograms.

constituted the crude extract of peroxidases linked ionically to the walls.

Peroxidase activities analysis

Peroxidase activities analysis was carried out according to the method of Thorpe et al. (1978). In 5 ml of reaction mixture (containing: 1 volume of H_2O_2 0.2% (V / V), 2 volumes of guaïcol 1% (V / V) and 5 volumes of phosphate buffer (1/15 M, pH 6.1)), the reaction was initiated by addition of 25 ml of crude enzyme extract. After homogenization and 5 min incubation, the peroxidase activity was determined by assaying tetraguaïacol (at 420 nm) formed from guaiacol using spectrophotometer (HITACHI U-2000). The peroxidase activity is expressed in term of optical density (OD) per min per mg of protein (in crude extract) and gram of the fresh of mesocrp of *Theobroma cacao* L.

To determine the isoenzymatic profile, native polyacrylamide gel electrophoresis of the peroxidase isoenzymes was carried out according to the modified Laemmli (1970) method. Briefly, the resolution gel was 10% and the stacking gel 6% (w / v). The electrode buffer was Tris (0.025 M) -Glycine (0.129 M), pH 8.3. The electrophoresis was carried out at a constant voltage of 300 V. After migration and demolding, the gel is rinsed with distilled water and the peroxidases are revealed by incubation in the benzidine-H_2O mixture prepared as follows (Table 1).

Estimation of the heterosis effect and heritability

Heterosis or hybrid vigour is estimated by comparing F1 hybrid vigour to the means of both parents (P1 and P2). This hybrid vigour (HF %) expressed as a percentage is calculated according to Gallais (1990) and Zahour (1992).

HF (%) = [F1-[(P1+P2)/2]/ [(P1+P2)/2]] ×100

The heritability (h2) of the different parameters measured was estimated according to the Falconer and Mackay formula (1996) .Heritability is the proportion of the phenotypic variance that is due to genetic variance. During this work, heritability in the narrow sense (h2) was estimated. According to the formula, h2 = Additive genetic variance / Phenotypic variance = Additive genetic Variance / Genetic Variance + Environmental Variance [h2 = VA / VP = VA / (VG + VE)].

VA=Additive genetic Variance
VG = Genetic Variance
VE = Environmental Variance
VP = Phenotypic Variance

Statistical analyzes

The data from this work is presented in the form of means ± SD. Analysis of variance (ANOVA) and comparison of averages by the Duncan test at $P < 0.05$ were performed using SPSS software version 12 for Windows at a 5% probability level to compare sensitivity levels and peroxidase activity in hybrid genotypes from reciprocal cross of parents. Hierarchical classification are performed using SPAD software version 4.1 for Windows.

RESULTS

Soluble POX

Variation in speciffic activities of the soluble POX

In the healthy pods, the specific activity of the soluble POX (SASPOX) is high in the T79 / 467 tolerant clone (29.02 ± 0.9 IU mg^{-1} of protein) and in the F1315 hybrid genotypes (50, 87 ± 2.32 IU mg^{-1} of protein), F1314 (40.03 ± 1.75 IU mg^{-1} of protein), F1313 (39.15 ± 0.43 IU mg^{-1} of protein) and F1307 (38.06 ± 0.3 IU mg^{-1} of protein). Under lesion conditions, the specific activity of the soluble POX increase in parents and in 100% of hybrid genotypes. These increases are greater in hybrid genotypes F1324 (33%), and F1321 (11%). Under inoculation conditions, the increases of this specific activity of the soluble POX are more important in the parents and in 100% of the hybrid genotypes studied. These increases are greater in clone T79 / 467 (81%) and hybrid genotypes F1307 (98%), F1314 (62%) and F1313 (52%) (Figure 2).

In the healthy pods of the progeny 79, the specific activity of the soluble POX is high in hybrids genotypes F7928 (38.66 ± 3.09 IU mg^{-1} of protein), F7902 (37.66 ± 1.73 IU mg^{-1} of protein) and F7907 (34.16 ± 1.73 IU mg^{-1} of protein).Under lesion conditions, the specific activity of the soluble POX increase in parents and in 88% of hybrid genotypes. These increases are greater in hybrids genotypes F7926 (50%) and F7928 (29%). Under inoculation conditions, the increases of this specific activity of the soluble POX are more important in parents and in 88% of hybrid genotypes. These increases are greater in

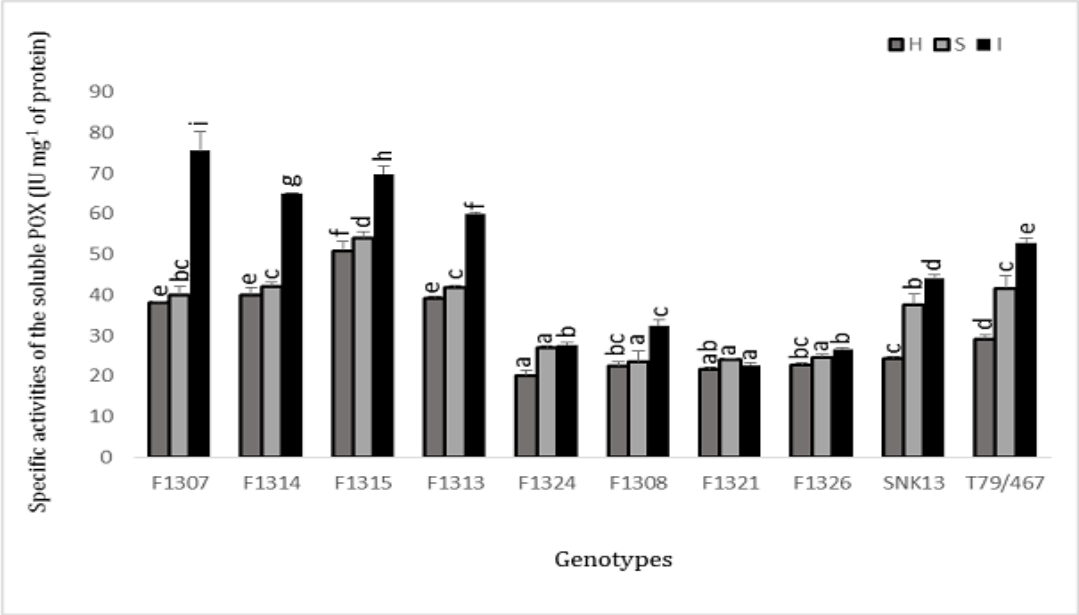

Figure 2: Variation of specific activity of the soluble peroxidase in parental and hybrid genotypes of the progeny F13 at different treatment conditions

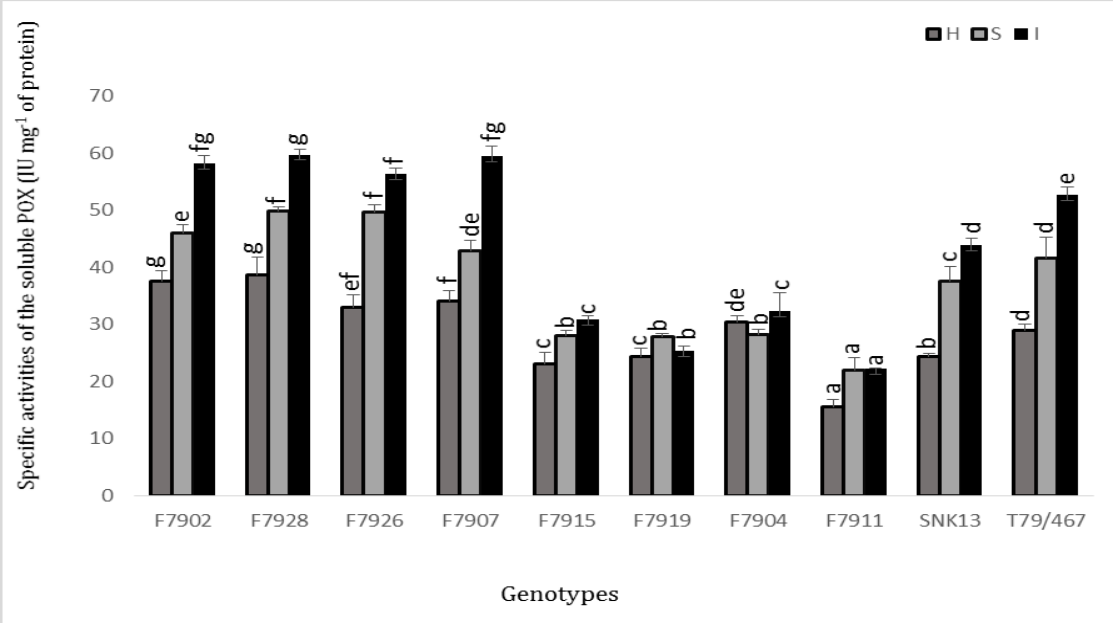

Figure 3: Variation of specific activity of the soluble peroxidase in parental and hybrid genotypes of the progeny F79 at different treatment conditions.

hybrid F7907 (74%) and F7926 (70%) (Figure 3).

Bonded POX

Variation in specific activities of the bonded POX

lesion conditions, the specific activities of the bonded POX

In the healthy pods, the specific activities of the bonded POX (SABPOX) varies little between parental clone T79 / 467 (17, 98 ± 0.91 IU mg^{-1} of protein) and parental clone SNK13 (14.67 ± 0.66 IU mg^{-1} of protein). In the progeny F13, the specific activities of the bonded POX are important in the hybrids genotypes F1313 (41.93 ± 1.26 IU mg^{-1} of protein) and F1315 (41.37 ± 1.98 IU mg^{-1} of protein). Under

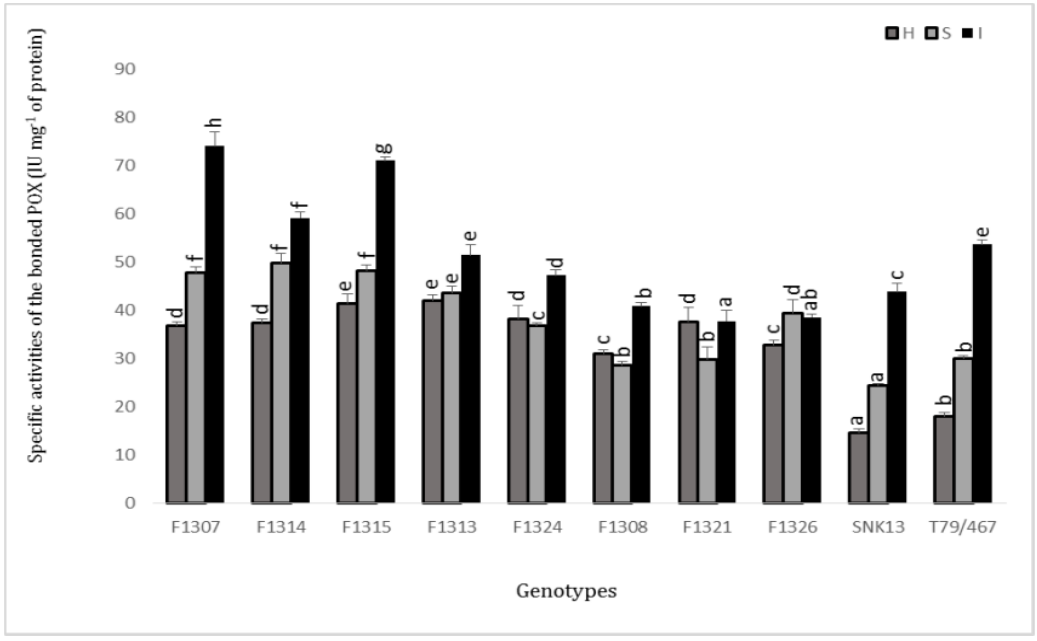

Figure 4: Variation of specific activity of the bonded peroxidase in parental and hybrid genotypes of the progeny F13 at different treatment conditions.

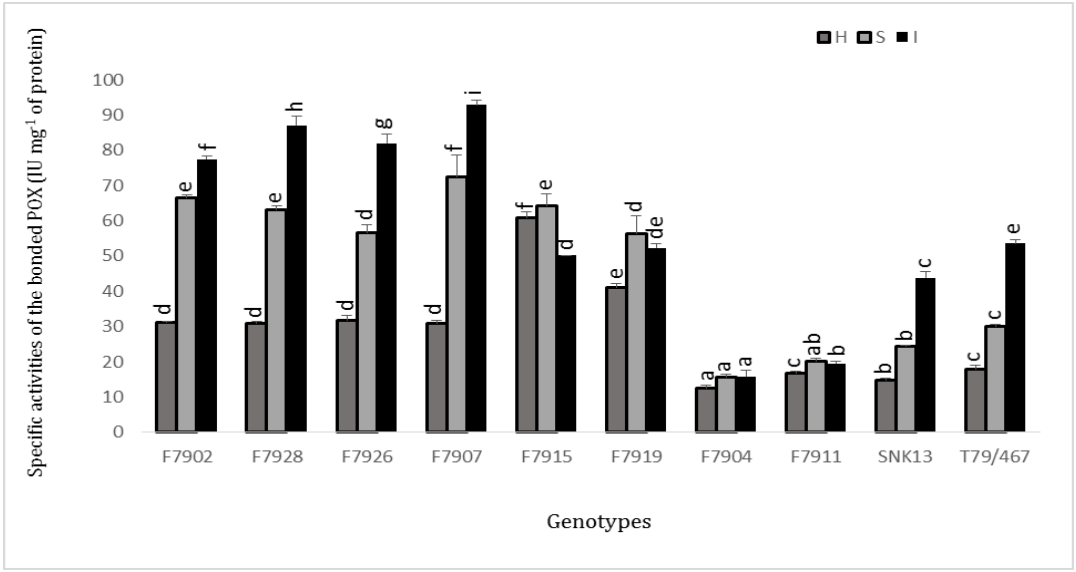

Figure 5: Variation of specific activity of the bonded peroxidase in parental and hybrid genotypes of the progeny F79 at different treatment conditions.

increase in parents and in 63% of the hybrid genotypes. These increases are greater in hybrid genotypes F1314 (33%) and F1307 (29%). Under inoculation conditions, the increases of the specific activities of the bonded POX are high in all hybrid genotypes studied. These increases are greater in hybrid genotypes F1307 (101%), F1315 (71%) and F1314 (58%) (Figure 4).

In hybrid genotypes of the progeny F79 in healthy, the specific activities of the bonded POX are high in the hybrid genotypes F7915 (60.74 ± 1.9 IU mg^{-1} of protein) and F7919 (41.08 ± 0.96 IU mg^{-1} of protein). Under lesion conditions, these specific activities of the bonded POX increase in parents and in 100% of individuals. These increases are greater in hybrid genotypes F7907 (134%), F7902 (113%), F7928 (105%) and F7926 (78%). Under inoculation conditions, the specific activities of the bonded POX are also increase in parents and in 100% of the hybrid genotypes studied. These increases are greater in hybrid genotypes F7907 (200%), F7928 (182%), F7926 (158%) and F7902 (148%) (Figure 5).

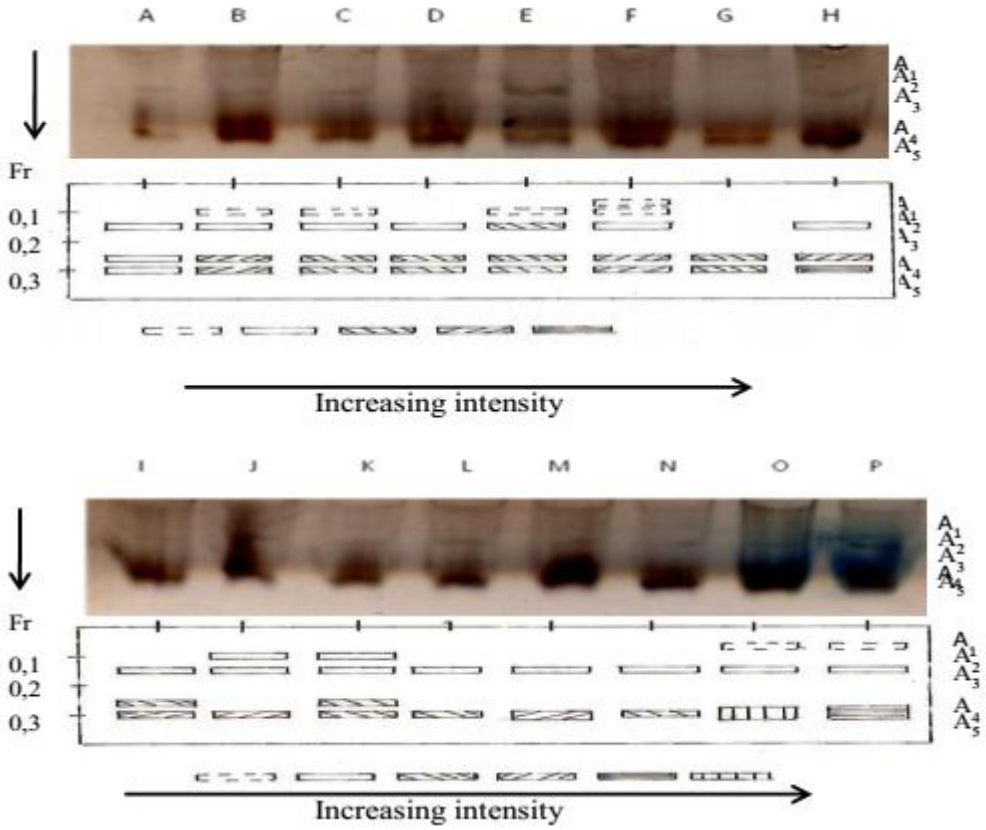

Figure 6: (a-b). Electrophorétic profiles of solubles POX in the cortex parental and hybrid genotype pods submitted at different treatments (Healthy, Scarified and Inoculated). A : Healthy T79/467; B : Scarified T79/467; C : Inoculated T79/467; D : Inoculated F7919; E : Inoculated F7926; F : Inoculated F7902; G : Inoculated F7911; H : Healthy F1315 ; I : Scarified F1315; J : Inoculated F1315; K : Inoculated F1307; L : Inoculated F1324; M : Inoculated F1321; N : Healthy SNK13; O : Scarified SNK13; P : Inoculated SNK13.

Electrophoretic profile of soluble peroxidases

The analysis of zygmograms obtained from soluble POX profiles shows four isoforms respectively in families F13 and F79. The sensitive and productive parental clone SNK13 in healthy condition reveals two isoforms (A3 and A5). Under lesion and inoculation three isoforms (A1, A3 and A5) are observed. The less productive and tolerant parental clone T79 / 467 revealed three isoforms (A3, A4 and A5). Under lesion and inoculation, four isoforms (A2, A3, A4 and A5) are observed.

In the progeny F13, the more tolerant and less productive F1315 genotype in healthy and lesion conditions reveal 3 isoforms (A3, A4 and A5). Under inoculation conditions, 3 isoforms (A2, A3 and A5) are also observed. In the more tolerant and productive hybrid genotype F1307 under inoculation conditions, 4 isoforms (A2, A3, A4 and A5) are observed. In the less tolerant and more productive hybrid genotype F1324 under inoculation conditions, 2 isoforms (A3 and A5) are observed. In the less tolerant and less productive hybrid genotype F1321 under inoculation conditions, 2 isoforms (A3 and A5) are also observed.

In the progeny F79, the more productive and more tolerant hybrid genotype F7902 under inoculation conditions, 5 isoforms (A1, A2, A3, A4 and A5) are observed. In the more tolerant and less productive hybrid genotype F7926, 4 isoforms (A2, A3, A4 and A5) are observed. In the less tolerant and more productive F7919 genotype, 3 isoforms (A3, A4 and A5) are observed. And in the less tolerant and less productive hybrid genotype F7911, 2 isoforms (A4 and A5) are observed (Figure 6).

Determination of heterosis effects of the soluble peroxidases

In the progeny F13, the manifestation of hybrid vigour was observed in all treatment conditions. 50, 12.5 and 50% of hybrid genotypes had positive values respectively in healthy, scarified and inoculated conditions in soluble POX. The manifestations of positive hybrid vigour was observed in hybrid genotypes F1315, F1307, F1314 and F1313 under inoculated conditions (Table 2).

Table 2. Heterosis Value (%) of hybrid genotypes of the progeny F13 of soluble POX

Genotypes	Treatments		
	Healthy	Scarified	Inoculated
F1307	+56.81	-9.86	+56.25
F1314	+64.93	-5.51	+34.62
F1315	+109.61	+21.28	+44.41
F1313	+61.31	-6.25	+23.91
F1324	-16.93	-39.23	-42.76
F1308	-7.90	-46.79	-32.71
F1321	-11.20	-45.68	-53.15
F1326	-5.84	-44.77	-45.17

Table 3. Heterosis Value (%) of hybrid genotypes of the progeny F79 of soluble POX

Genotypes	Treatments		
	Healthy	Scarified	Inoculated
F7902	+55.17	+3.42	+20.51
F7928	+59.30	+12.39	+23.75
F7926	+35.87	+11.66	+16.63
F7907	+40.77	-3.38	+23.29
F7915	-5.22	-36.81	-36.26
F7919	+0.23	-37.54	-47.49
	+25.66	-36.43	-53.762
F7911	-35.99	-36.26	-54.04

In the progeny F79, the manifestation of hybrid vigour was also observed in all treatment conditions. 75, 37.5 and 50% of hybrid genotypes had positive values respectively in healthy, scarified and inoculated conditions. The highest vigour values were observed in hybrid genotypes F7928, F7902, F7907 and F7926 under inoculated conditions (Table 3).

Hierarchical classification of hybrid genotypes

The soluble PSA obtained in the different treatment conditions made it possible to carry out the hierarchical classifications of the hybrid genotypes of the different progenies. A grouping of parental and hybrid genotypes was achieved at 95% homogeneity.

For progeny F13

The direct hierarchical classification of the genotypes taking into account all the treatment conditions makes it possible to distinguish 3 groups. The first was made of the hybrid genotypes F1321 and F1326 which are characterized by low specific activities of the soluble POX. The second group was made of the parents SNK13, T79 / 467 and the hybrid genotype F1324. The third group consists of the hybrid genotypes F1315, F1313, F1314 and F1307 which are characterized by high specific activities of the soluble POX.

Taking into account the inoculation conditions only, three groups of different configuration for the second and the third groups are also distinguished. The second group was made of parents SNK13, T79 / 467 and hybrid genotypes F1324 and F1308. The third group includes the hybrid genotypes F1313, F1314, F1315 and F1307 (Figure 7).

Progeny F79

The direct hierarchical classification carried out from the specific activity of the soluble POX under all treatment conditions had presented three distinguish groups. The first was made of the hybrid genotypes F7911 and F7904 which were characterized by low specific activities of the soluble POX. The second made of the sensitive parent SNK13 and the hybrid genotypes F7915 and F7919 which were characterized by average specific activities of the soluble POX meanwhile the third group constituted of tolerant parent T79 / 467 and hybrid genotypes F7907, F7926, F7928 and F7902 were characterized by high specific activities of the soluble POX. Under inoculation conditions only, three similar groups of the same hybrid genotypes were also distinguished (Figure 8).

Heritability of specific activity of the soluble POX

Strict heritability values (h2) of the specific activity accumulation of the soluble POX were estimated by evaluating the ratio of Additive genetic Variance to Phenotypic Variance. The heritability values obtained in the reciprocal progenies F79 (♀T79 / 467 x ♂SNK13) and F13 (♀SNK13 x ♂T79 / 467) were not very different. The heritability values of the reciprocal progenies F13 (0.65) and F79 (0.62) showed a strong correlation between the specific activities of the soluble POX of parents and those of the progenies.

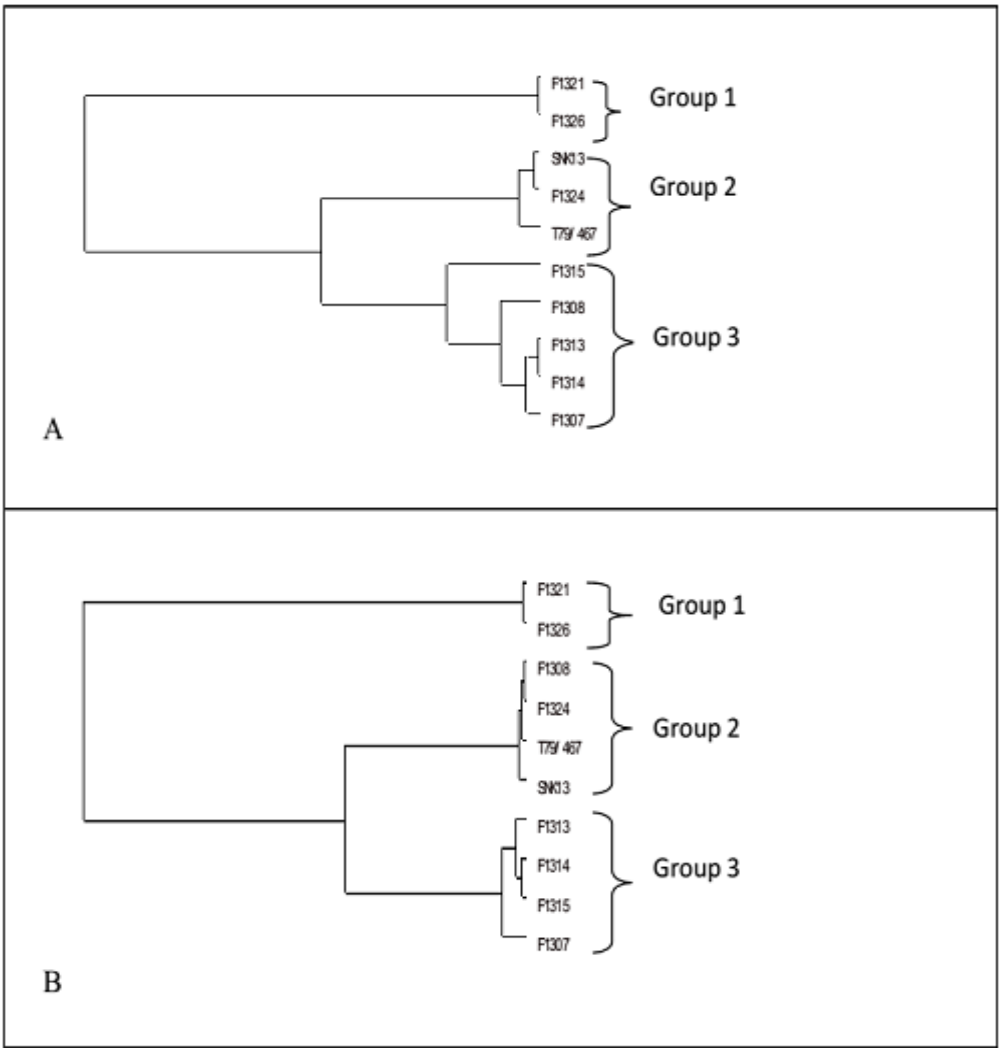

Figure 7: Direct hierarchical classification of hybrid genotypes of progeny F13 using specific activities of the soluble POX in all treatment conditions (A) and under inoculated conditions (B).

Correlations between necrosis and specific activity of the soluble and bonded POX

The exploitation of the necrosis surfaces and the specific activities of the soluble and bounded POX showed the different correlations obtained. In the progeny F79, the correlation was negatively significant ($P < 0.01$) between necrosis and specific activity of the soluble POX on the one hand and on the other hand, between necrosis and specific activity of the soluble and bonded POX (Table 4). In the progeny F13, the the similar results ($P < 0.01$) between the necrosis and the specific activities of the soluble and bounded POX were obtained (Table 4).

DISCUSSION

The transmission of soluble and bound peroxidases from reciprocal crosses ♀SNK13 x ♂T79 / 467 was evaluated. In general, the specific activity content of peroxidases extracted at pH 7 is higher in the soluble fraction than in the ionically bound fraction in parent clones and in both progenies. In healthy pods, heterogeneity is observed in different hybrid genotypes relative to their peroxidase content. Thus, constitutively, the specific activity levels of the peroxidases do not make it possible to predict the susceptibility level of *T. cacao* with respect to *P. megakarya*. However, it is well known that the products of the so-called resistance genes activate a cascade of events initiating the response of the plant to the aggressions (Cassab, 1998; Essam et al., 2014). These enzymes are usually expressed in the healthy plant. Following a parasitic attack, the interaction with the gene products of the pathogen allows these enzymes to acquire a specific conformation, which allows them to trigger the defense response (Hammond-Kosack and Jones, 1997). In addition, whatever the hybrid genotype studied, there is a strong accumulation of specific activities of soluble peroxidases under stress conditions.

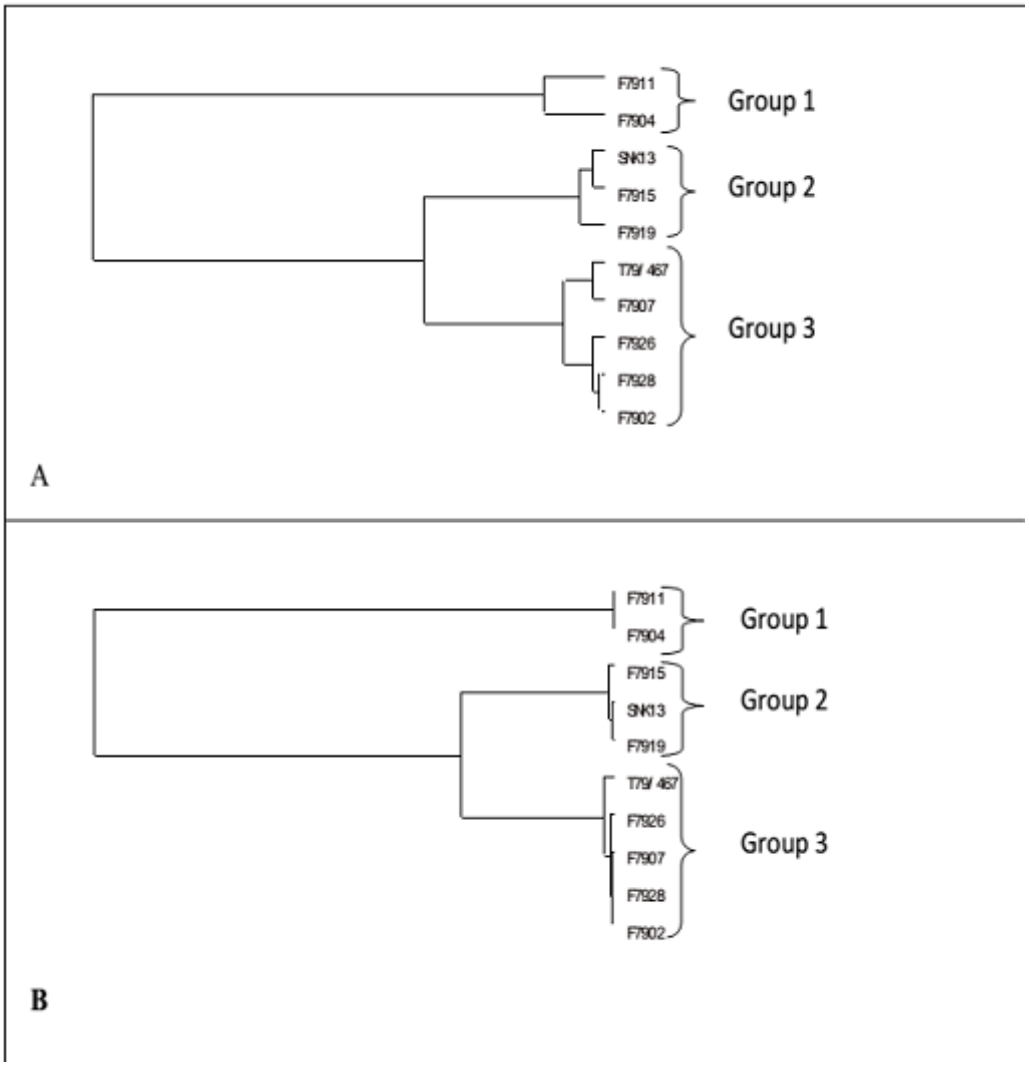

Figure 8: Direct hierarchical classification of hybrid genotypes of progeny F79 using specific activities of the soluble POX in all treatment conditions (A) and under inoculated conditions (B).

Table 4. Correlations between the necrosis and evaluated in families F79 and F13

	Progeny F79			Progeny F13		
	Necrosis	SASPOX	SABPOX	Necrosis	SASPOX	SABPOX
Necrosis	1			1		
SASPOX	-0.596**	1		-0.754**	1	
SABPOX	-0.593**	0.824**	1	-0.769**	0.737**	1

* Significant correlation at the 0.05 level; ** Significant correlation at the 0.01 level.

This increase is more significant in inoculation compared to the stress of simple scarification (Essam et al., 2014). However, this accumulation does not depend on the susceptibility of the different hybrid genotypes studied. Such observations were made by Nemec (1995) in cucumber studies.

Many authors in previous studies have shown the involvement of peroxidases in plant resistance. They have shown that these enzymes accumulate in the plant inoculated with avirulent or virulent pathogens (Housti et al., 2002; Wititsuwannakul et al., 2002). The data presented in this study show a relationship between the specific activity of peroxidases and resistance stimulation in cocoa against *P. megakarya*. After inoculation of the pods, there is a strong increase of the specific activity of these enzymes in the soluble and ionically bound fractions in the more tolerant hybrid genotypes in both progenies. In fractions (S), this increase is greater in the progeny F13 where there

is an increase of 144%. On the one hand, in the fractions (L), it is more important in the progeny F79 where there is an increase of 200%. At the same time, there is a correlation between the rate of increase of specific peroxidase activities and the degree of tolerance of the genotypes in fractions (S) and (L) within the two hybrid progenies. On the other hand, these activities do not correlate with productivity. These results are similar to those obtained by Omokolo et al. (2003) on the same plant and those obtained by Mbouobda et al. (2010) who observed an accumulation of POX after inoculation of Xanthosoma sagittifolium roots with Benza (1, 2, 3) thiodiazole-7-carbothioic S-methyl ester (BTH). These results are also similar to those obtained by Delledone et al., 2002, which showed during the study of the resistance response to hypersensitive plant diseases that peroxidases play a key role in the local and systemic resistance of plants infected.

Delannoy et al. (2003) also showed an increase in POX activity in cotton tissues under inoculation conditions. Indeed, POX are involved in the plant defense mechanism and the increase in activity in response to inoculation can be correlated with the pathosystem and the level of host resistance (Delannoy et al., 2006). More tolerant and more productive hybrid genotypes (F1307, F1314, F7902, F7928) and more tolerant hybrid genotypes (F1315, F1313, F7926, F7907) compared to the best parent in soluble and bound fractions record large amounts of specific activities of POX in these fractions with small areas of necrotic lesion in contrast to less tolerant and productive genotypes (F1324, F1308, F7915 and F7919) that accumulate small amounts of specific activities of POX in these fractions with large areas of necrotic lesion and thus show the involvement of POX in tolerance / resistance and not in productivity. These results are in agreement with those obtained by Manga et al., 2016, which showed after infection of *Theobroma cacao* leaves by the mycelium of *Phytophthora megakarya* that the specific activities of POXs increase significantly in condition of scarification and infection in tolerant hybrid genotypes. They have shown that these enzymes act as a barrier against the invasion of pathogens and are therefore part of the host's tolerance / resistance mechanism. These results are also similar to those obtained by Khales and Baaziz (2006) who showed a strong accumulation of specific POX activities in the ecotypes of opuntia ficus indicate under stress conditions.

The profile of the peroxidase isoforms (S) of the mesocarp of the inoculated pods reveals the existence of a specific form (A2) after inoculation in the tolerant hybrid genotypes T79 / 467, F7902, F7926, F1315 and F1307. This isoform is related to tolerance. These observations have been made by Esnault and Chibbar (1997) who have shown the appearance of new isoperoxidases related to inoculation in tobacco. However, the appearance of a new isoform (A1) common to the tolerant parenting genotype (T79 / 467) and to the susceptible genotype (SNK13) in the inoculation conditions suggest rather a differential differentiation of this enzyme between the different tissues of the pod (Kombrink and Somssich, 1997). The results also show that isoperoxidases are present in both healthy pods and pods treated. They are therefore constitutive and have no significant correlation neither with sensitivity to *Phytophthora megakarya* nor with productivity. This suggests a regulation of the expression of this enzyme during the development of the pod. The electrophoretic profiles of POX showed that the increase of their activity after inoculation is due to the activation of pre-existing isoforms. POX can therefore be used as markers of tolerance / resistance of plants in several abiotic or biotic constraints (Khales and Baaziz, 2006).

In general, POX is responsible for the rapid accumulation of quinones in tissues. Quinones are involved in the fight against infection. This explains the increase of their activities coupled with those of the other existing enzymes in the plant. In the palm, Baaziz (1992) has shown that the co-existence of a POX activity associated with other enzymes increases the oxidative power of these enzymes. The response to inoculation is therefore reflected by the increase in POX activities and those of the other enzymes of the plant. This tolerance / resistance mechanism seems to involve the activation of lignification and the production of toxic quinones for the fungus. The manifestation of hybrid vigour is important in both progenies. In addition, the heritability values of the specific activities of POX obtained in fractions (S) in the progeny F13 (♀SNK13 x ♂T79 / 467) and in the progeny F79 (♀T79 / 467 x ♂SNK13) are relatively high. They are 0.65 and 0.62 respectively. These high values show a strong additive variance in the transmission of tolerance / resistance to black pod disease. The manifestation of hybrid vigour and heritability values inherited from the offspring indicate a good overall ability to combine parental clones. These results are similar to those obtained by some authors who have shown that the heritability of tolerance / resistance of *Theobroma cacao* to *Phytophthora* species is mainly controlled by additive genetic effects with an additive-dominated genetic model (Adomako, 2006; Nyadanu et al., 2017). Such observations have also been described by Djocgoue et al. (2007) after studies of phenolic compounds on *T. cacao* leaves. All these observations indicate the strong involvement of POX in the heritability of tolerance / resistance to black pod disease, which suggests the use of these markers to assist selection patterns in cocoa. The negative and significant correlation between the development of necrosis and the soluble and bound peroxidase activities in the two progenies suggests an implication of these enzymes in this plant's tolerance / resistance mechanism against pathogen attack. These results are in agreement with those obtained by Manga et al., 2016, which obtained similar results after infection of the leaves of the same plant by the pathogen.

Conclusion

The evaluation of the heterosis effect of POX in pods of *Theobroma cacao* made it possible to compare and classify

the different hybrid genotypes of progenies F13 and F79. This physiological parameter was examined in healthy, scarified and inoculated pods. The evaluation of the heritability of POX reveal high and similar values in both progenies show that these enzymes are heritable and are therefore transmitted from parents to offsprings. This work has also made it possible, on the one hand, to note that POX activity is colored at the tolerance / resistance level and not at the productivity level in both the soluble fractions and the bound fractions and on other hand that the profile of the peroxidase (S) isoforms of the mesocarp of infected pods revealed the existence of a specific isoform (A2) after infection in the tolerant hybrid genotypes T79 / 467, F7902, F7926, F1315 and F1307. This specific isoform would be related to tolerance. The existence of this specific isoform (A2) of tolerant hybrid genotypes could be used in the creation of more productive and tolerant varieties. These varieties with interesting characteristics could be vulgarized to farmers in order to enable them to increase yields on their cocoa plantations and consequently the increase of their purchasing power.

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Conflict of interests

The authors declare that they have no conflicting interests.

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