Myrosinase and cysteine in *Theobroma cacao* L. defense mechanism against *Phytophthora megakarya*

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Abstract

Develop *T. cacao* hybrid genotypes tolerant to *P. megakarya* is the way out to improve cocoa production and profitability in cocoa producing countries. Hence, the analysis of susceptibility to *P. megakarya* of the progeny ♀SNK64x♂UPA143 was conducted for the first time. Additionally, cysteine content and myrosinase activity were analyzed in healthy, wounded and wounded_infected leaves in other to bring out the implication of cysteine and myrosinase in *T. cacao* defense against *P. megakarya*. The screening for susceptibility to *P. megakarya* of the progeny ♀SNK64x♂UPA143 has revealed that, more than 58 % of the progeny ♀SNK64x♂UPA143 displayed negative Mid-Parent Heterosis (*MPH*) and around 79 % were less sensitive to black pod disease than the most sensitive parent UPA143. This set of results indicates that the progeny ♀SNK64x♂UPA143 might be recommended for development of *T. cacao* hybrid genotypes tolerant or less sensitive to black pod disease. Cysteine content in healthy leaves significantly decrease in wounded leaves. The decrease in cysteine content was amplified when wounded leaves were infected. Myrosinase activity presented reverse pattern compared to cysteine profile in healthy, wounded and wounded_infected leaves. This finding might indicate that, during the abiotic or biotic stress, cysteine might be used for the synthesis of glucosinate. And the synthesized glucosinolate might be hydrolyzed by myrosinase to

liberate metabolites which might protect *T. cacao* against abiotic or biotic (due to *P. megakary* infection) stress. This is supported by the fact that Myrosinase activity increase with abiotic and biotic stress. This could indicate that, in *T. cacao* defense against abiotic or biotic stress, there would be existed specific myrosinase isoform (s) associated to tolerance. Hence, ♀SNK64 and♂UPA143 could be used to produce tolerant hybrid genotypes for cocoa famers in other to improve profitability. Additionally, cysteine and myrosinase profiles could be useful in *T. cacao* breeding.

Key words: Hybrid, breeding, defense, cocoa, myrosinase, amino acid.

Introduction

Cocoa (*T. cacao*) cultivation is an important component of Cameroon agricultural sector and plays a crucial role in its economy since pre-independence period till now. As one of the main cash crops, lives of 2.4 - 4.2 million of Cameroonians are directly depended on cocoa cultivation. However, Cameroun faces difficulties to substantially increase its cocoa production which ranks between 180,000 tones (2000/2001) and 250,000 tones (2015/2016) [1]. This quasi stagnation in cocoa production is mainly due to cocoa planting material crisis. There is a lack of reliable planting material for a sustainable cocoaculture. Frequently used planting material are highly sensitive to the most destructive cocoa disease, black pod disease due to an oomycete, *Phytophthora megakarya* [2] This pathogen can cause 50 to 100 % cocoa production lost depending to the cocoa genotype, *P. megakarya* strain and season despite the ongoing cultural methods [3][4] Knowing the negative environmental incidences of chemical when used against plants pathogens, enormous efforts are now focused in development of genetically tolerant genotypes using cross-pollination of target parent's and early selection of progeny with interesting traits [5][6]. The efficiency of early selection of elite progeny is relied on adequate test used. Leaf disc test analysis has been studied by [7] in the same plant in laboratory and the results from these studies

showed the correlation with field observations [8]. Further investigations used the same leaf disc analysis but there were some unmatchable observations. It appears therefore necessary to combine leaves disc test analysis with biochemical markers of plants defense against phytopathogens. The combination of leaves disc test with defense biochemical markers analysis would add accuracy and reliability in selection of elite hybrids. Additionally, biochemical makers would be helpful in breeding.

Myrosinase (EC 3.2.3.1) is a β-thioglucosidase mainly reported in all *Brassicaceae* not yet reported in *T. cacao* [9]. The enzyme hydrolyzes various sulfur-containing glucosides known as glucosinolates [9]. The hydrolysis of glucosinolate by β-thioglucosidase release products with various biological activities depends on the structure of glucosinolate side chains and plant species. However, regarding their potential role in plants, the most accepted opinion is that the glucosinolate-myrosinase system plays an important role in defense against pathogens [10][9]. But this is not yet studied nor reported in *T. cacao*. Metabolically, thioglucosides derive from sulfur containing amino acids such as methionine which sulfur moiety is from cysteine, the platform of sulfur metabolism in plant [11]. Sulfur and sulfur derivatives are reported to be implicated in plants defense. Oilseed rape resistance against *Leptosphaeria maculans*, *Botrytis cinerea* and *Phytophthora brassicaein* was reported to be closed link to sulfur statute of the plant [12]. Hence, sulfur containing molecules can be associated to *T. cacao* selection for tolerance against *P. megakarya*. The present work aims to study the implication of cysteine and myrosinase in *T. cacao* defense against *P. megakarya* and their potential use in the selection of tolerant hybrids of *T. cacao*.

Material and methods

Plant material and experiment design

A nursery was set up using seeds from cocoa pods obtained by manual crossed-pollination of ♀SNK64x♂UPA143 at seeding farm of

Barombi Kang (Kumba, South-West – Cameroon). Leaves from four months old plantlets (hybrids) derived from the above cocoa pods seeds were used as plant material for subsequent studies.

Plantlets from full sib progeny from ♀SNK64x♂UPA14 were randomly placed in three blocks in the nursery (7mx8m, 14hr light/10hr darkness at ≈ 26±1 ºC). Partially lignified leaves (2.5 months old) from each hybrid were submitted to leaf discs test in triplicate using adjusted method of [7]. The experimental design was made of triplicates and completely randomized 5 blocks of leaf discs (Ø = 1.5 cm) per hybrid. A total of 21 discs were used per hybrid

Zoospore production

Zoospores (or inoculums) were obtained from l0-days-old cultures of *P. megakarya* moderated virulent strain according to [7] adapted method. Cultures with sporangia were induced to liberate zoospores by adding sterile distilled water at 4°C. After 1 hour at room temperature, the zoospore concentration was adjusted to $3.l0^5$ zoospores/mL with a MALASSEZ hemati-meter.

Screening for susceptibility of ♀SNK64x♂UPA143 progeny to *P. megakarya*

Three leaves from each hybrid of the progeny were harvested early in morning, washed successively with tape and distilled sterilized cool water prior to leaf discs collection. Seven leaf discs per leaf were obtained from the slightly lignified young leaves. Leaf discs were placed in trays and incubated for 24 hours (at 25±1 ºC) in darkness prior to inoculation. After the 24 hours, leaf discs were inoculated by depositing 10 µL ($3.l0^5$ zoospores/ml) of zoospores suspension on either side in the middle of each leaf disc and incubated in darkness (at 25±1 ºC). The necrosis rate or disease score (from 0: "tolerant" to 5: "highly sensitive") of susceptibility (through the necrosis size) of each leaf discs (for each hybrid) was registered on 4^{th}, 5^{th}, 6^{th}, 7^{th} and

8th day after inoculation. This experiment was done in triplicates.

Soluble cysteine extraction and analysis

Leaves of 2.5 months old were slightly grounded in a mortar in the presence of 5 mL acetone (to remove chlorophyll) and dry for 5 min in room temperature on filter paper Wattman Nº1. Then, 0.5 g of chlorophyll-free leaves was ground in presence of 2.5 mL of ethanol 80º and centrifuged for 30 min at 6000g. The supernatant was collected for cysteine quantification.

Cysteine content was determined according to [13] method. Cysteine extract (0.15 mL) was mixed with 0.35 mL of acidic ninhydrine reagent [1.3 % (w/v) ninhydrine in 1:4 concentrated HCl: CH3COOH]. The mixture was heated at 100 °C for 10 min then cooled in ice bath to allow ping color development. The optical density was read at 560 nm against the control in which the 0.15 mL of cysteine extract was replace by equal volume of ethanol 80º.

Myrosinase extraction and assay

A mass of 0.1 g of chlorophyll-free leaves was ground in 2.5 mL of Tris-HCl 50 mM (pH 7.5) buffer containing 3 mM DTT, 1 mM EDTA and 5 % (v/v) glycerol and a pinch of polyvinylpyrrolidone. The mixture was then centrifuged (10 000g, 30 min, 4 °C) and the supernatant (protein extract) was collected for protein quantification according to [14] method. Myrosinase activity was evaluated in each protein extract from different *T. cacao* hybrids using adjusted method of [15]. Myrosinase activity was determined by measuring the formation of glucose subsequently to degradation of sinigrin (Sigma, St. Louis, USA). The reaction mixture was incubated at 35 °C for 5 min before adding the protein extract. Glucose release after 10 min incubation was determined using Müller reagent. The optical density was read at 575 nm and the myrosinase activity was reported in $mmol.min^{-1}.mg^{-1}$ protein.

Heterosis estimation

The heterosis rate (*HF*) was estimated from the formula of [16] as follows:

Mid-parent heterosis (MPH) = [Fi - MP] /MP

Best-parent heterosis (BPH) = [Fi -BP]/BP

Where: Fi = hybrid value; MP = the mid-parent value of both parents; BP = value of the best parent.

Data analysis

Statistical analyses were performed by SPSS (version 17.0) software. Data were subjected to descriptive statistic and analysis of variance (ANOVA). The standard deviation was estimated and the means were separated with the test of Student, Newman and Keuls (SNK) at 5% significance level. Spearman correlation analysis between variables was performed.

Table 1. Germination rate (%) of seeds

Weeks	Germinated seeds (%)	Not germinated seeds (%)
1st	53.70	46.30
4th	88.89	11.11
8th	90.13	9.87

Results

a) Germination rate

Seeds from cocoa pods obtained by manual pollination of ♀SNK64 x ♂UPA143 were used to set up a nursery. Germination rates were: 53.70%, 88.89 % and 90.13% respectively 1st, 4th and 8th weeks after seeding (Table 1).

Screening for susceptibility of the progeny ♀SNK64 x ♂UPA143

Disease scores of the progeny ♀SNK64x♂UPA143 were monitored from day 4 to day 8 after leaf discs inoculation. These data were submitted to Student Newman and Keuls test. It has been observed an increase of the number of susceptibility subgroups of hybrid genotypes with a peak at day 7. At this date, twelve hybrids genotypes susceptibility subgroups

or disease scores phenotypic classes were obtained: [0, 0.4[, [0.4, 0.8[, [0.8, 1.2[, [1.2, 1.6[, [1.6, 2.0[, [2.0, 2.4[, [2.4, 2.8[, [2.8, 3.2[, [3.2, 3.6[, [3.6, 4.0[, [4.0, 4.4[and [4.4, 4.8]. The most tolerant subgroup of hybrids genotypes with phenotypic disease score ([0, 0.2[) exhibited the highest percentage of hybrids genotypes 20.83 %. The most susceptible subgroup of hybrids genotypes ([4.4, 4.8[) has exhibited low percentage hybrids genotypes (8.33%). More than 60 % of hybrids genotypes exhibited a disease score lower than mean phenotypic disease score value (2.4) of the progeny ♀SNK64 x ♂UPA143 (Table 2).

At day 7 of leaf discs inoculation, about 20.83 % of hybrid genotypes exhibited disease scores lower than the most tolerant parent SNK64 (0.3±0.01) and, only 20.83 % of hybrids showed a disease score higher than the most susceptible parent UPA143 (3.8±1.2). When compared to the reference clone SCA6 (disease score 0.1± 0.01) 12.5 % of ♀SNK64x♂UPA14 offprint were more tolerant to black pod disease than the reference clone.

Analysis of Best Parent Heterosis (*BPH*) and Mid-Parent Heterosis (*MPH*)

Best parent heterosis (*BPH*) rates were estimated from the most tolerant parent SNK64. And Mid-Parent Heterosis (*MPH*) rates were estimated from the mean scores disease of both parents (SNK64 and UPA143). At day 7 of leaves discs inoculation, about 33.33 % of the offprint exhibited positive *BPH* rates. Positive *BPH* rates were ranging between -1 and -0.2. At the same date of leaves discs inoculation, 66.67 % showed a negative *BPH* rates while negative heterosis rates were ranged from 1 to 3.8. Around 45.83 % of the progeny showed positive Mid-parent heterosis (*MPH*) at day 7 of leaves discs inoculation (Table 3).

Table 2. Comparison of hybrids and parents disease scores using Student-Newman-Keuls test

	Days			
	4	5	6	7
Hybrid genotypes	Disease scores			
F6419	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
F6424	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
F6404	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
F6408	0.0^{a}	0.0^{a}	0.2^{a}	0.2^{ab}
F6414	0.0^{a}	0.0^{a}	0.2^{a}	0.2^{ab}
F6421	0.0^{a}	0.0^{a}	0.0^{a}	0.4^{ab}
F6418	0.0^{a}	0.4^{ab}	0.4^{a}	0.4^{ab}
F6406	0.0^{a}	0.0^{a}	0.2^{a}	0.8^{abc}
F6412	0.0^{a}	1.0^{ab}	2.0^{bcd}	2.0^{cde}
F6410	0.0^{a}	0.0^{a}	1.0^{ab}	1.2^{abc}
F6401	0.0^{a}	1.2^{abc}	2.0^{bcd}	2.0^{cde}
F6407	0.2^{a}	0.2^{a}	1.0^{ab}	1.6^{bcd}
F6403	0.8^{ab}	1.8^{bcd}	2.4^{bcdef}	2.8^{defg}
F6413	0.8^{ab}	1.8^{bcd}	2.0^{bcd}	2.6^{def}
F6420	1.0^{ab}	1.4^{abc}	1.4^{abc}	1.6^{bcd}
F6417	1.2^{abc}	1.4^{abc}	1.4^{abc}	2.0^{cde}
F6409	1.6^{abcd}	2.4^{cde}	2.8^{cdef}	3.4^{efgh}
F6415	2.0^{bcde}	2.0^{bcd}	2.2^{bcde}	2.8^{defg}
F6411	2.0^{bcde}	2.8^{def}	2.8^{cdef}	3.2^{efgh}
F6422	2.4^{cde}	3.0^{def}	3.6^{ef}	4.4^{hi}
F6405	2.6^{de}	3.2^{ef}	3.2^{def}	4.0^{fghi}
F6416	2.6^{de}	3.8^{f}	3.6^{ef}	4.8^{i}
F6423	2.8^{de}	3.6^{ef}	3.8^{f}	4.2^{ghi}
F6402	3.0^{de}	3.6^{ef}	3.6^{ef}	4.0^{fghi}
Parental clones				
SNK64	**0.0^{a}**	**0.0^{a}**	**0.1^{a}**	**0.3^{ab}**
UPA143	**3.2^{e}**	**3.6^{ef}**	**3.7^{f}**	**3.8^{fghi}**
Reference clone				
SCA6	**0.0^{a}**	**0.0^{a}**	**0.0^{a}**	**0.1^{ab}**

Necrosis rating was zero (none) to five (true necrosis). Necrosis rates at 4, 5, 6 and 7th day of leaves discs incubation are expressed in term of Means ± SD (n= 21). Mean of necrosis rates affected with the same letter (in a giving column) are not significantly different according to the Student, Newman and Keuls test at 5%. In hybrids column: reference genotypes (underline); parents' genotypes (in bold).

Biochemical analysis

a) Cysteine content

Cysteine content was analyzed in healthy, wounded and wounded_infected leaves from

some hybrids of the progeny ♀SNK64x♂UPA143. In healthy leaves, variability in cysteine content was observed between hybrid genotypes. Cysteine contents varied from 67.67±13.6 µg/g FW in F6405 to 685.0±30.8 µg/g FW in F6401 hybrid genotype. Globally, the wounding of leaves was associated to a significant decrease in cysteine content in leaves. The gap of cysteine decrease (compared to healthy leaves) was hanging between 17 and 76.66 % except for hybrids. Additional and substantial decrease (compared to healthy and wounded leaves) in cysteine content in leaves was observed when wounded leaves were infected. Differences in cysteine content between wounded and wounded_infected leaves were above 80 % (Fig.1).

b- Correlation between disease scores, cysteine contents in healty, wounded and wounded_infected leaves.

Pearson correlation was analyzed between disease scores, cysteine contents in healty, wounded and wounded_infected leaves. It appeared that, there was negative and significant correlation between disease scores and cysteine contents in wounded leaves. A negative correlation was also observed between disease score and cysteine content in wounded_infected leaves. Additionally, positive and highly significant correlations were noted between cysteine contents in wounded and wounded_infected leaves (Table 4).

c- Myrosinase activity

Myrosinase specific activity was analyzed in healty, wounded and wounded_infected leaves of four hybrid genotypes. It appears that the specific activity of this enzyme was sensitive to leaves stress. An abiotic stress due to wound induced an increase of myrosinase activity in *T. cacao* leaves. A substantial increase in myrosinase activity was observed when wounded leaves were infected. Hence, in the four hybrid genotypes, there is an increase of myrosinase activity from healthy to infected leaves (Fig. 2).

Table 3. Heterosis rates of the progeny ♀SNK64 x ♂UPA143

	Days					
	4	5	7	4	5	7
Hybrids	*BPH*			*MPH*		
F6419	-1	-1	-1	-1.0	-1.0	-1.0
F6424	-1	-1	-1	-1.0	-1.0	-1.0
F6404	-1	-1	-1	-1.0	-1.0	-1.0
F6408	-1	-1	-0.8	-1.0	-1.0	-0.9
F6414	-1	-1	-0.8	-1.0	-1.0	-0.9
F6421	-1	-1	-0.6	-1.0	-1.0	-0.8
F6418	-1	-0.6	-0.6	-1.0	-0.8	-0.8
F6406	-1	-1	-0.2	-1.0	-1.0	-0.6
F6412	-1	0	1	-1.0	-0.5	0.0
F6410	-1	-1	0.2	-1.0	-1.0	-0.4
F6401	-1	0.2	1	-1.0	-0.4	0.0
F6407	-0.8	-0.8	0.6	-0.9	-0.9	-0.2
F6403	-0.2	0.8	1.8	-0.5	-0.1	0.4
F6413	-0.2	0.8	1.6	-0.5	-0.1	0.3
F6420	0	0.4	0.6	-0.4	-0.3	-0.2
F6417	0.2	0.4	1	-0.3	-0.3	0.0
F6409	0.6	1.4	2.4	-0.1	0.2	0.7
F6415	1	1	1.8	0.2	0.0	0.4
F6411	1	1.8	2.2	0.2	0.4	0.6
F6422	1.4	2	3.4	0.4	0.5	1.1
F6405	1.6	2.2	3	0.5	0.6	1.0
F6416	1.6	2.8	3.8	0.5	0.9	1.3
F6423	1.8	2.6	3.2	0.6	0.8	1.0
F6402	2	2.6	3	0.8	0.8	1.0

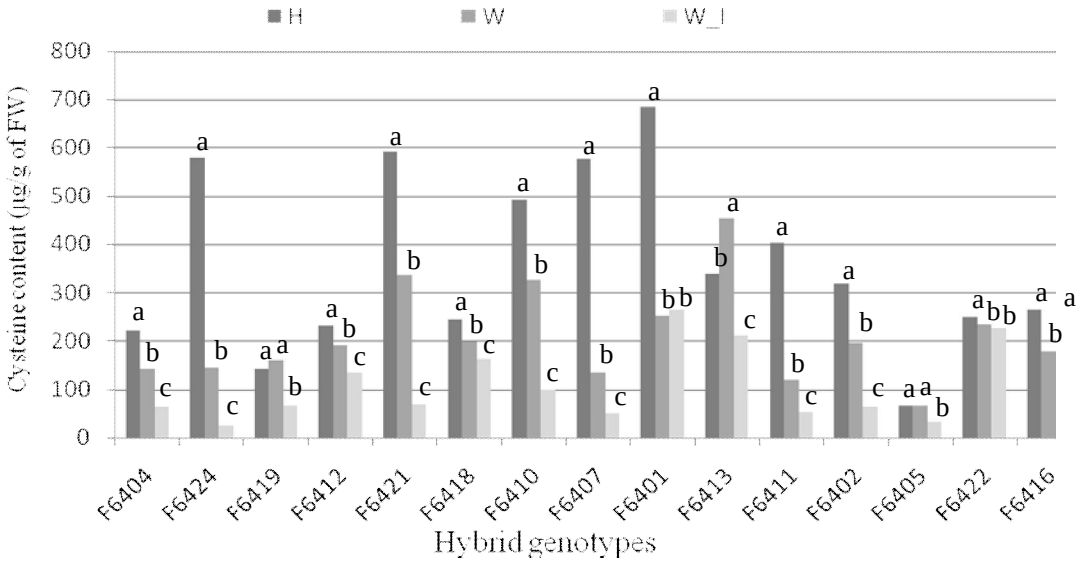

Fig. 1. Cysteine contents in healty, wounded and wounde_infected leaves of *T. cacao* hybrid genotyes. Treatments affected with the same letter (in a giving hybrid genotype) are not significantly different according to the Student, Newman and Keuls test at 5%. H = healthy leaves, W= wounded leaves, W_I = wounded and infected leaves.

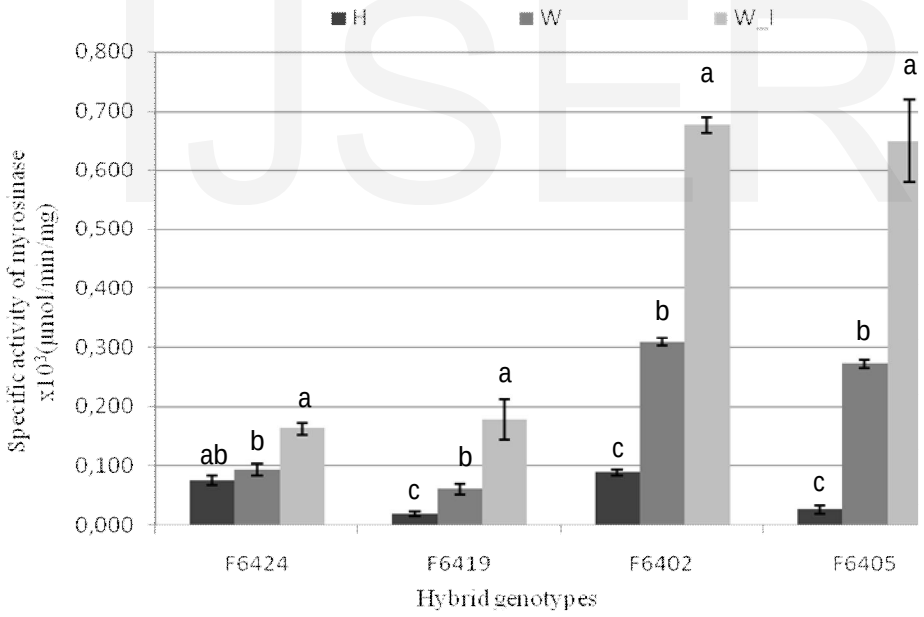

Fig. 2. Specific activity of myrosinase in healty, wounded and wounded_infected leaves of *T. cacao* hybrid genotypes. Treatments affected with the same letter (in a giving hybrid genotype) are not significantly different according to the Student, Newman and Keuls test at 5%. H = healthy leaves, W= wounded leaves, W_I = wounded and infected leaves.

Discussion

Manual pollination between ♀SNK64 and ♂UPA14 was conducted with 37 % success. Matures pods derived-seeds were able to germinate (90.13 %) when used to set up a nursery. Leaves from above seeds-derived plantlets were submitted to screening for black pod disease susceptibility, cysteine and myrosinase analyses (in healty, wounded and wonded_infected leaves).

Screening for black pod disease susceptibility of progeny ♀SNK64x♂UPA14 revealed variability in the distribution of disease scores among the hybrid genotypes. This observation might indicate that the progeny ♀SNK64x♂UPA14 is heterozygote for black pod disease susceptibility character. This finding bring out the fact that *T. cacao* clones used in cross-pollination are always heterozygote for their sensitivity to back pods disease [8]. The number of phenotypic subgroups of hybrid genotypes susceptibility and the variability of percentage of hybrid genotypes per phenotypic subgroup might reveal the polygenic character of *T. cacao* susceptibility to black pod disease [17][18].

In cross-pollination of many *T. cacao* genotypes, [19] reported that the proportion of tolerant hybrid from a giving cross-pollination did not excide 1/3. The evaluation of heterosis showed that more than 58 % and 20.8 % of the progeny ♀SNK64x♂UPA143 presented negative *MPH* and *BPH* respectively. Furthermore, around 79 % of ♀SNK64x♂UPA14 progeny were less sensitive to black pod disease than the most sensitive parent UPA143. Additionally, some hybrid genotypes exhibited disease scores lower than a reference clone SCA6 which is known as tolerant to black pod disease. Hence, the progeny ♀SNK64x♂UPA143 might therefore be recommended for development of *T. cacao* hybrid genotypes tolerant or less sensitive to black pod disease. The high proportion of tolerant hybrid genotypes of this progeny (♀SNK64x♂UPA143) might derive from the female parent SNK64, which is reported to be tolerant to black pod disease [20][6]. Additionally, the tolerant/ resistant

character of *T. cacao* hybrid genotypes seems to be female-parent-dependant and the transmission of this character is additive [21].

Cysteine contents analysis was conducted in healthy, wounded and wounded_infected leaves from hybrid genotypes. Globally, cysteine contents in healthy leaves from hybrid genotypes were variable. However, the cysteine contents in healthy leaves appeared to be not associated to sensitivity of hybrid genotypes. When healthy leave were wounded there was a significant decrease in leaves cysteine content. The decrease in leaves cysteine contents was amplified when wounded leaves were infected. These set of data might reveal firstly the mobilization and the use of cysteine when *T. cacao* is exposed to abiotic (wound) and biotic (*P. megakary* infection) stress. The decrease of cysteine contents might indicate that cysteine is used under abiotic and biotic stress. In fact, cysteine might be used in the biosynthesis of anti-stress metabolites such as the tripeptide, glutathione, the major thiol compound in most eukaryotes [22].

The mobilization of cysteine under abiotic and biotic stress might reveal that, this sulfurous amino acid intervene in defense mechanism of *T. cacao* against *P. megakary*. The importance of cysteine in defense mechanism of *T. cacao* against *P. megakary* might be laid on the thiol (-SH) moiety of its radical. The –SH moiety of cysteine is known to be used in the biosynthesis of plant sulfur-rich defense compounds such as glucosinolate [11][23][24]. Hence, the observed negative and none significative correlation between disease score and cysteine contents in wounded or wounde_infected leaves could result from indirect implication cysteine in the defense system of *T. cacao* against *P. megakary*. Furthermore, Sulfur-containing metabolites are reported to be responsible for sulfur induced resistance in many plants [25][26][27].

Myrosinase activity level in healthy leaves appeared to be hybrid genotypes-dependent. Additionally, the activity of this

β-thioglucosidase (EC 3.2.3.1) was affected by wound and infection of leaves. Myrosinase might be therefore implicated in *T. cacao* defense under abiotic and biotic stress.

Our results have indicated the increase of myrosinase activity from healthy to wounded leaves and from wounded to wounded_infected leaves. Myrosinase activity pattern in healthy, wounded and wounded_infected leaves appeared therefore to be reverse to cysteine pattern in the same plant material (healthy, wounded and wounded_infected leaves). In fact, myrosinase is known as glucosinolate-degrading enzyme. The sulfur moiety of glucosinolate derives from cysteine, the distributor platform of organic sulfur in plant cell [11]. Hence, the reverse patterns between myrosinase and cysteine profiles during abiotic or biotic stress could be laid on metabolic pathways. During the abiotic or biotic stress, cysteine might be used for the synthesis sulfur-containing-anti-stress metabolites such as glucosinate. And the synthesized glucosinolate might be hydrolyzed by myrosinase to liberate metabolites which might protect *T. cacao* against abiotic or biotic (due to *P. megakary* infection) stress. In our result, the level of myrosinase activity was not associated to disease scores (susceptibility statute) of tested hybrid genotypes. This could indicate that, in *T. cacao* defense against abiotic or biotic stress, there would be existed specific myrosinase isoform(s) associated to tolerance.

Conclusion

The cross-pollination of ♀SNK64 and ♂UPA143 generates a high proportion of tolerant hybrid genotypes which could be used by cocoa famer for a sustainable cocoaculture and profitability. Fundamentally, when *T. cacao* is exposed to *P. megakarya*, cysteine is used for the synthesis of sulfur defense molecule such as glucosinolates which is hydrolyzed to release defense melabolites against *P. megakarya*. The set of information are useful in T. cacao breeding.

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