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Assessment of three cocoa hybrid families' susceptibility and cysteine involvement in defense process against *Phytophthora megakarya*

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Black pod disease (BPD) is the major cocoa pathology constraint caused by an Oomycete, *Phytophthora megakarya* prevailing in African cocoa producing countries. The development of *T. cacao* planting material tolerant to BPD lies on cross-pollination of adequate parental genotypes which generate offspring with desirable traits. We assessed the susceptibility to BPD of offspring derived from three manual crosses (SNK13xUPA143, T79/501xUPA143 and UPA143xSNK64) using leaf discs test (LDT) which evaluates disease scores of hybrid genotypes. Cysteine involvement in *T. cacao* defence process against BPD has been studied for the potential use of this sulphur amino acid profile to identify tolerant cocoa hybrid genotypes. LDT displayed variable disease scores patterns. Within a given family, LDT revealed heterogeneity in disease scores. This heterogeneity may have been derived from polygenic character of *T. cacao* susceptibility to BPD. In SNK13xUPA143 family, 26.13% exhibited disease scores lower than two (tolerant hybrid genotypes). T79/501xUPA143 and UPA143xSNK64 generated 21.18 and 26.46% of tolerant hybrid genotypes respectively. This variability between families may have resulted from deferential susceptibility of parental clones; UPA143 being more susceptible than T79/501 followed by SNK13. SNK64 was the less susceptible to BPD. The percentages of tolerant hybrid genotypes in this study appeared to be consistent hence, SNK13xUPA143, T79/501xUPA143 and UPA143xSNK64 could be used to produce hybrids genotypes tolerant to BPD. Cysteine analysis was performed in none detached healthy and wounded-infected leaves of hybrid genotypes with variable disease scores. It appeared that cysteine was mobilized during the infection. Two main patterns were observed: in tolerant hybrid genotypes (disease score lower than 2), infection was associated to a significant reduction of cysteine content in young leaves while in susceptible hybrid genotypes, infection was associated to an increase (accumulation) of cysteine in young leaves. Cysteine could be involved in the synthesis of effective defence molecules against *P. megakarya* in tolerant hybrid genotypes. This set of finding may indicate that cysteine profile could be used to discriminate tolerant from susceptible hybrid genotypes of *T. cacao* to *P. megakarya*.

Key words: Cocoa tree, black pod disease, hybrids genotypes, tolerance, cysteine.

INTRODUCTION

Chocolate tree (*Theobroma cacao* L.) is a tropical rainforest plant widely cultivated in Africa, America and Asia (Alverson et al., 1999; Whitlock et al., 2001; Acebo-Guerrero et al., 2012; Ngoh Dooh et al., 2015). In Cameroon, cocoa is one of the most economically important crops grown by small farmers for their livelihoods (Sonwa et al., 2008; ICCO, 2013, 2015). However, there is approximately 50 - 80% of cocoa production harvest losses because of an oomycete, *Phytophthora megakarya*, the pathogen of black pod disease in Cameroon (Djocgoue et al., 2010; Mfegue, 2012). Classically, pesticides are used to reduce the incidence of black pod disease (BPD). However, these chemicals appeared to be environmentally unfriendly, expensive, and inefficient (Sonwa et al., 2008). Hence, breeding for tolerance to BPD appeared to be the way out for sustainable cocoa culture (Iwaro et al., 2005; Ramalho et al., 2012; Bohinc and Trdan, 2012). For this, genetic improvement of cocoa through generative strategy is mostly recommended (Tahi et al., 2000; Nyassé et al., 2003; Ondobo et al., 2014; Effa et al., 2015). Generative breeding is based on the aptitude of a giving couple of parents (genotypes or clones) to generate offspring with desirable traits (Eskes and Lanaud, 1997). Obviously, many couples have not yet been assessed in this purpose, such as SNK13xUPA143, T79/501xSNK13 and UPA143xSNK64

SNK64 clone is the less susceptible to BPD, followed by SNK13, T79/501 (obtained from P7xNa32) and UPA143 clones. UPA143 and T79/501 are upper Amazonians clones, known for their high productivity but very low tolerance to *P. megakarya*. SNK13 and SNK64 belong to Trinitario group of cocoa. SNK13 is a moderately susceptible and productive clone while SNK64 is the most tolerant among the four clones but low yielding (Nyasse et al., 2007). Cross pollination between the above clones might generate offspring with interesting agronomic traits (resistance/tolerance to BPD).

Biomolecules associated to *T. cacao* defence against pathogens could also be useful in the development of *T. cacao* hybrid genotypes tolerant to BPD such as sulfur which has been used as a most important fungicide used especially for antifungal treatments of fruits (Tweedy, 1981; Hassall, 1990; Manga et al., 2016).

Nowadays, industries are producing fungicides with specific and diverse modes of action. Elemental sulfur has always been used because of the development of resistance against the compounds with unique site of action (Jolivet, 1993). Many studies have reported the involvement of sulfur in the defense mechanisms of plants. However, sulfur defense mechanisms are still unclear (Saito, 2000; Cooper and Williams, 2004; Hamdan, 2010). This might involve elemental sulfur form, or organic-sulfur-containing compounds directed against microorganisms (Cooper and Williams, 2004; Hamdan, 2010; Stanislaus, 2011). In *T. cacao*, elemental sulfur was reported to be involved in the protection of this plant against *Verticillium dahliae* (Cooper et al., 1996; Cooper and Williams, 2004). Sulfur moiety in organic sulfur containing compounds in plants is mainly provided by cysteine. Cysteine is the first organic molecule in the reduction/assimilation sequence of inorganic sulfur (SO_4^{2}). Cysteine is considered as the hub of sulfur distribution in organic molecules including those involved in the defense system of plants against biotic stress (Saito, 2000). Hence, cysteine profile might be a useful biochemical marker in the selection of *T. cacao* hybrid genotypes tolerant to *P. megakarya*.

This investigation aimed to: (a) assess the susceptibility to BPD of offspring derived from SNK13xUPA143 (KHA), T79/501xSNK13 (AHK) and UPA143♀xSNK64 (AHK3) and (b) study the profile of cysteine (variability in cysteine contents) during *T. cacao* / *P. megakarya* interaction in order to select tolerant hybrid genotypes which could be distributed to farmers.

MATERIALS AND METHODS

Plant material

Leaf samples from the offspring derived from the crosses SNK13 X UPA13 (KHA), T79/501 X SNK13 (AHK) and UPA143 X SNK64 (AHK3) were used for the study. Manual pollination was conducted in the seeding farm of the Institute of Agricultural Research for Development (IRAD) at Barombi-Kang (Kumba, South-West Region, Cameroon, Africa).

T. cacao seeds from mature pods derived from ♀SNK13x♂UPA143, ♀T79/501x♂SNK13 and ♀UPA143x♂SNK64 were used to set up a nursery. Leaves of three to four months old seedlings from SNK13xUPA143, T79/501xSNK13 and UPA143xSNK64 (of the above nursery) were used for the screening for susceptibility using leaf disc test and cysteine profile analysis.

P. megakarya strain culture

The Plant Pathology Laboratory of IRAD (Nkolbison, Yaoundé, Centre region, Cameroon, Africa) provided the strain of *P. megakarya* with moderate virulence. Virulence was maintained by artificial inoculation of pods followed by the re-isolation and transfer in the phenylethyl alcohol agar (PEA) medium. This was also used to induce the release of zoospores, which were used in the leaf discs test.

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Figure 1. Disease score of leaves infected *in plantae* in the nursery. A =0 ; B=+ ; C =++ ; D=+++ ; E= ++++; F ++++++; 1 cm = 4 cm.

Zoospores productions

Zoospores (or inoculums) were obtained according to Tondje et al. (2006) method. Zoospores were produced by the inoculation of pods (with *P. megakarya* mycelia) followed by soaking them in sterile distilled water when sporangia recover infected pods. The solution was placed at +4°C for 5 min then immediately transferred in room temperature to allow sporangia to liberate zoospores. The zoospores concentration was adjusted to $3x10^5$ zoospores/mL with a MALASSEZ hemati-meter.

Screening for susceptibility to *P. megakarya* and experimental design for leaves discs test

Two to three months old leaves were harvested (three leaves/plant) between 6 and 7 am. Using a cork cutter, 8 discs of 1.5 cm diameter were collected per leave. Hence a total of 24 discs were obtained per leave. These discs were placed bottom-side turned upward, on a wet trays and incubated for 24 h in darkness at 25 ± 1°C prior to inoculation. After the 24 h, leaf discs were inoculated by depositing 10 µL ($3x10^5$ zoospores/ml) of zoospores suspension on either side, in the middle of each leaf disc and incubated in darkness (at 25±1°C). The scoring (from 0: tolerant to 5: highly sensitive) of susceptibility (through the necrosis size) of each leaf discs (for each hybrid) was registered on 4th, 5th, 6th, 7th and 8th day after inoculation.

Artificial inoculations of leaves in the nursery

The test of undetached leaves has been used in several studies to evaluate the tolerance of plants against *Phytophthora* (Ahmad Kamil et al., 2004; Djocgoue et al., 2006; Nyadanu et al., 2013). In our investigations, inoculation was made on none detached leaves in the nursery. The underside of the leaf was cleaned with cotton soaked in 70° ethanol. Scarification was made at the midrib, on which, 3 mm diameter mycelia-agar-disc of 10 days old culture was placed. Then covered with sterile cotton, protected plaster and moisturized with sterile water. The incubation time was 7 days. The disease score was arbitrary defined from 0 to 5 (Figure 1).

Extraction and quantitative analysis of the soluble cysteine

The inoculated nursery leaves were harvested 6 days after inoculation (DAI), with the controls (not inoculated) lightly ground in 5 ml acetone (to remove chlorophyll) and dried for 5 min at room temperature on Wattman Nº1 paper. 0.5 g of chlorophyll-free leaves was ground in 2.5 ml of ethanol 80°. Homogenate was subsequently centrifuged for 30 min at 6000 *g*. The supernatant was collected for cysteine quantification.

Cysteine content was determined according to Gaitonde (1967) method. Cysteine extract (0.15 ml) was mixed with 0.35 ml of acidic ninhydrin reagent [1.3 % (w/v) ninhydrin in 1:4 concentrated HCl: CH_3COOH]. The mixture was heated at 100°C for 10 min then cooled in ice bath to allow pink color development. The optical density was read at 560 nm ($\varepsilon = 2.8 \times 10^4$) against the control in which the 0.15 ml of cysteine extract was replaced by equal volume of ethanol 80°. Cysteine content was expressed in µg per gram of acetonic powder (µg/gFP).

Data analysis

Data collected were subjected to descriptive statistics. Analysis of variance (ANOVA) and mean separation by the Student-Newman-Keuls were done with SPSS 17.0 software. Discrimination of hybrid genotypes according to their disease scores (level of sensitivity) were done through direct hierarchical classification of the same software.

RESULTS AND DISCUSSION

Germination rate

Germination of seeds from mature pods derived from ♀UPA143x♂SNK64 (AHK3), T79/501xSNK13 (AHK) and SNK13xUPA143 (KHA) families were monitored during the 12 days after seeding. KHA family germination rate appeared to be lower than AHK and AHK3 families. At day 4 after seeding, 10 and 22% of seeds derived from AHK and AHK3 respectively germinated. At the same date, there was no germination of KHA seeds. At day 12, AHK family seeds exhibited germination rate of 91.49% while AHK3 showed 98.9% and the germination rate of KHA family was 40.35% (Table 1).

Assessment of hybrids susceptibility to *P. megakarya*

The susceptibility of F1 hybrids from the biparental

Table 1. Seed germination rate.

Germination rate	Hybrid family		
	KHA	AHK	AHK3
Germination rate at day 4 (%)	0	10	22
Germination rate at day 12 (%)	40.4	91.49	98.9

crosses (SNK13xUPA143, T79/501xUPA143 and UPA143xSNK64) to *P. megakarya* was monitored through disease score on the fourth, sixth and seventh day after leaf discs inoculation and incubation. The sixth day appeared to be most discriminative (by generating the highest number of phenotypic subgroups of disease scores) compared to the fourth and seventh day of leaves discs inoculation and incubation.

Family UPA143xSNK64 (AHK3)

At the sixth day of leaves discs inoculation, the 44 plantlets from the subfamily ahk3ca (of AHK3) displayed disease scores between 0.0±0.0 and 4.87±0.37. The 44 plantlets were separated in 18 phenotypic subgroups of disease scores using the Student Newman and Keuls test. Direct hierarchic classification regrouped the 18 phenotypic subgroups of disease scores in 7 classes: [0.0; 1], [1; 1.5], [1.5; 2.1], [2.1; 3], [3; 4], [4; 4.5] and [4.5; 5]. Approximately, 38.64% of hybrid genotypes from ahk3ca displayed disease scores in the interval [0.00, 1.9 (Figure 2 and Table 2).

In the same conditions, the subfamily ahk3cb displayed 13 phenotypic subgroups of disease score. The 13 phenotypic subgroups of disease scores were regrouped in fives classes using direct hierarchic classification: [0.0; 1.0 [(6.81%); [1.0; 2](4.54%) [2; 3] (9.10%), [3; 4] (25%) and [4; 5] (54.54%) (Figure 3 and Table 2). Disease scores ranged between 0.0±0.0 and 5.0±0.0. In addition, 11.37% of the offspring exhibited disease scores in the interval [0.00, 1.9].

Family T79/501xUPA143 (AHK)

The subfamily ahkca (of the family AHK) displayed 14 phenotypic subgroups of disease scores from 46 plantlets when data of disease scores were subjected to Student, Newman and Keuls test. The hierarchic classification test grouped the 47 plantlets in 6 classes: [0; 0.5[, [0.5; 1.75[, [1.75; 2.75], [2.75; 3.25], [3.25; 4] and [4; 4.5] (Figure 4 and Table 3).

The same analysis conducted with the subfamily ahkcb made of 38 plantlets generated 14 phenotypic subgroups of disease scores. The hierarchic classification analysis grouped the 38 plantlets in 9 classes. Disease scores of this subfamily were included in the interval [0.00, 4.38]. About 17.4% of offspring showed disease score value lower than 2 (Figure 5 and Table 3).

Family KHA (SNK13xUPA143)

At day six of leaves disc inoculation and incubation, disease scores values ranged from 0.12 to 5 in khaca subfamily (SNK13xUPA143). The Student, Newman and Keuls test reveals 9 phenotypic classes of disease score. Approximately, 11.76% of hybrid genotypes showed disease scores lower than 2 (Figure 6 and Table 4).

In khacb subfamily (SNK13xUPA143) Student, Newman and Keuls test displayed 8 phenotypic subgroups of disease scores grouped in 6 classes using the hierarchic classification analysis (Figure 7 and Table 4).

Biochemical analysis

Cysteine content was analyzed in undetached healthy (untreated) and wounded-infected leaves in target hybrid genotypes from the three families. For each family, chosen hybrid genotypes differed in their disease scores.

In the AHK3 family, AHK3ca21, AHK3ca24, AHK3ca25, AHK3cb1, AHK3cb20, AHK3ca1, AHK3ca39, AHK3ca35, AHK3cb9, AHK3ca36, AHK3ca18, AHK3ca7, AHK3cb4, AHK3cb7, AHK3ca17 and AHK3ca46 hybrid genotypes leaves (healthy and wounded-infected) were used for cysteine quantification. In healthy leaves, variability in cysteine content was observed between tested hybrid genotypes. When healthy leaves are wounded and infected, two patterns were observed: in hybrid genotypes with disease scores lower than 2.3 (AHK3ca21, AHK3ca24, AHK3ca25, AHK3cb1, AHK3cb20, AHK3ca1 and AHK3ca39), infection appeared to be associated with cysteine content decrease (compared to healthy leaves). Percentages of cysteine contents decrease ranged between 27.94 (AHK3cb20) and 74.90% (AHK3ca24). When disease scores were above 2.3, infection led to significant increase in cysteine contents (Figure 8a and 8b). Moreover, Pearson correlation analysis showed a positive and highly significant correlation between difference in cysteine contents (between healthy and wounded-infected leaves) and disease scores of hybrids genotypes (Table 5).

Cysteine contents in AHK and KHA families showed two patterns as observed in AHK3 family. In AHK, hybrid

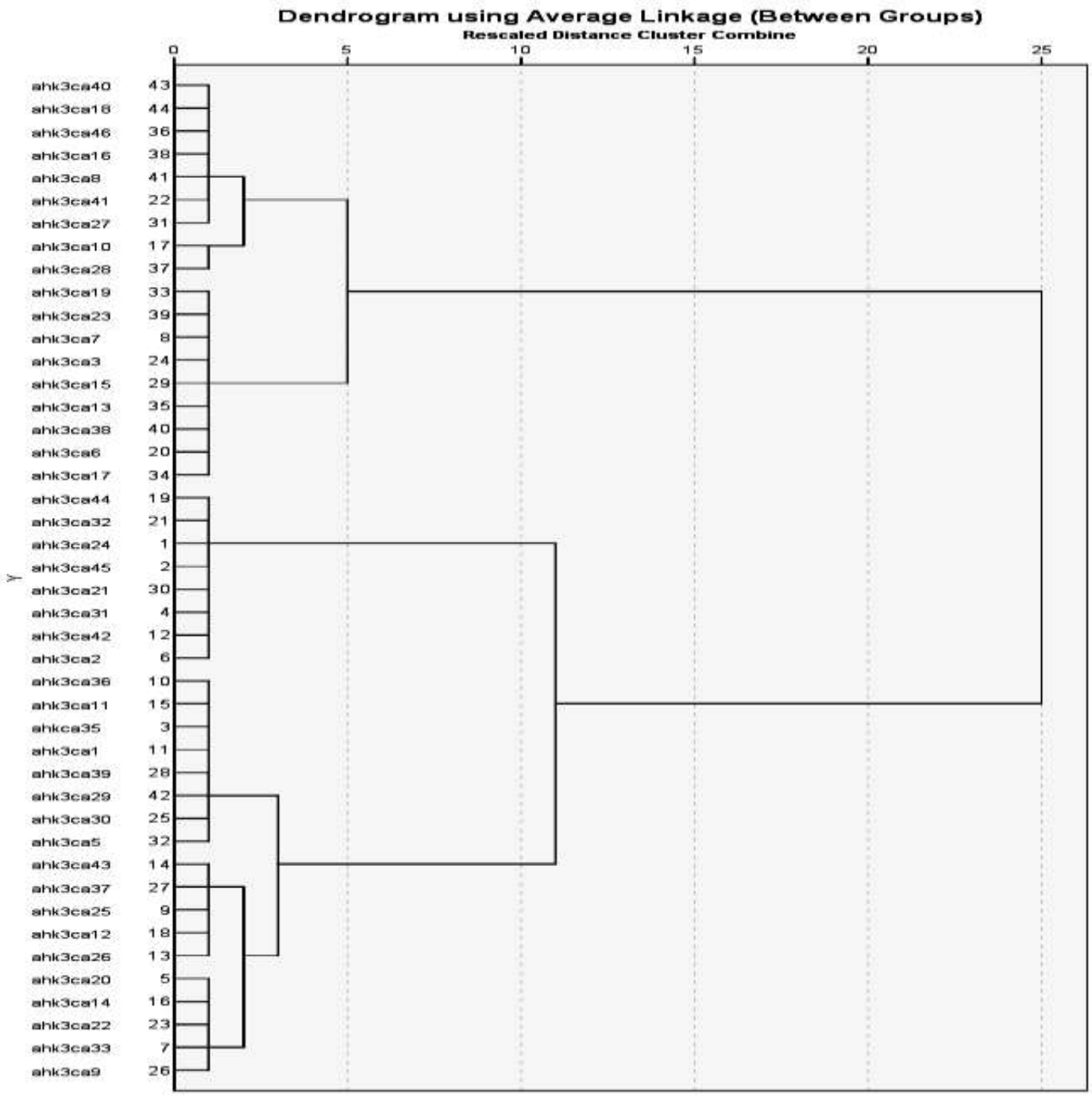

Figure 2. Direct hierarchic classification of hybrid genotypes of the subfamily ahk3ca (UPA143xSNK64).

Table 2. Phenotypic classes of disease scores of the family UPA143xSNK64 (AHK3).

Subfamilies	Phenotypic classes of disease scores (% of hybrid genotypes)						
	0; 1	**1; 1.5**	**1.5; 2**	**2; 3**	**3; 4**	**4; 4.5**	**4.5; 5**
ahk3ca	18.18	11.36	11.36	18.18	20.45	15.91	4.55
ahk3cb	6.82	4.55	0.00	9.09	25.00	18.18	36.36
Means	12.50	7.95	5.68	13.64	22.73	17.05	20.45

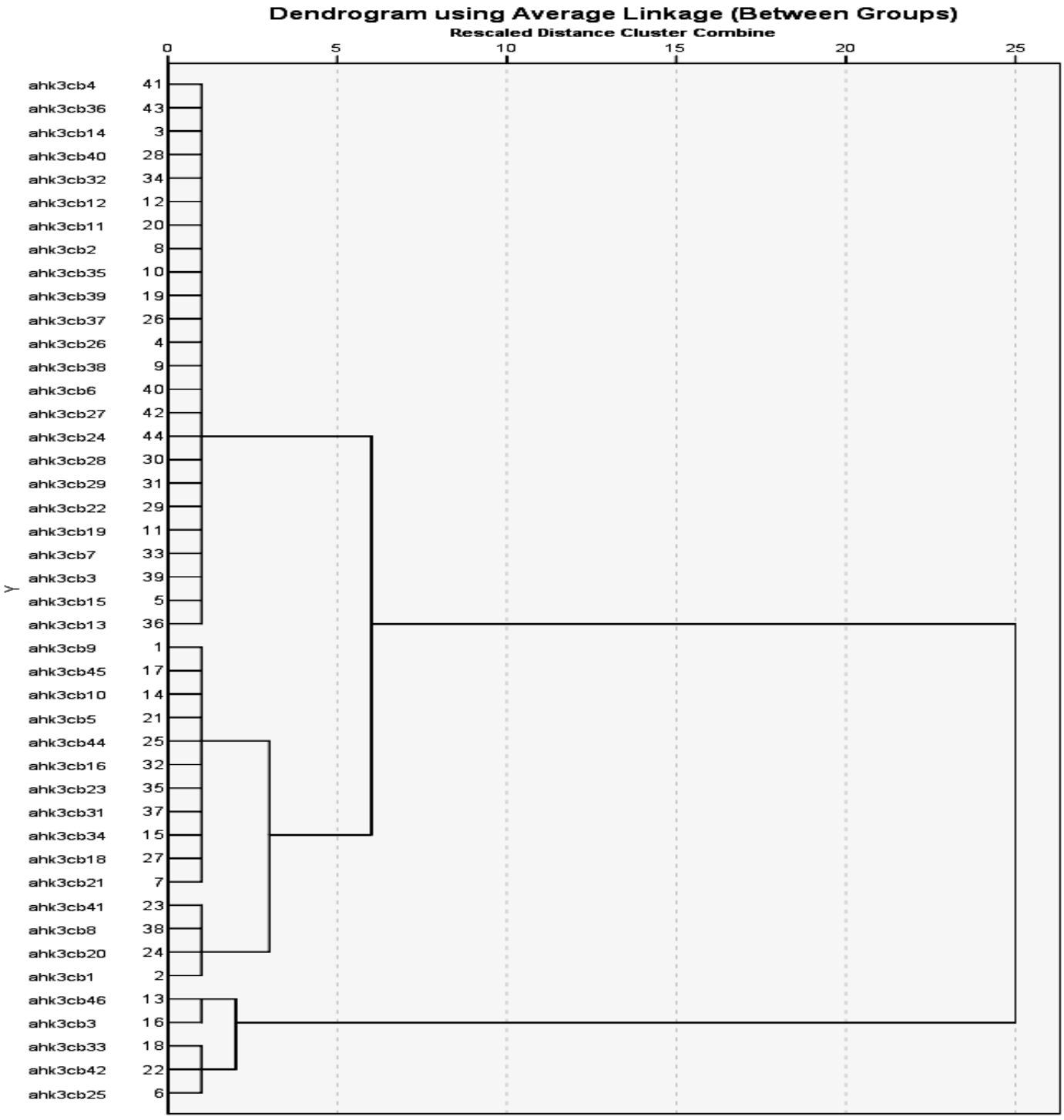

Figure 3. Direct hierarchic classification of hybrid genotypes of the subfamily ahk3cb (UPA143xSNK64).

genotypes with disease scores lower than 3.5; leaves infection led to decrease of cysteine contents (Figure 9a and 9b). In KHA family, hybrid genotypes with disease score under 3.7 exhibited decrease in cysteine content when leaves were infected (Figure 10).

DISCUSSION

A nursery has been established from the crosses pod seeds: SNK13xUPA143, T79/501xSNK13 and UPA143xSNK64. The involvement of cysteine in the

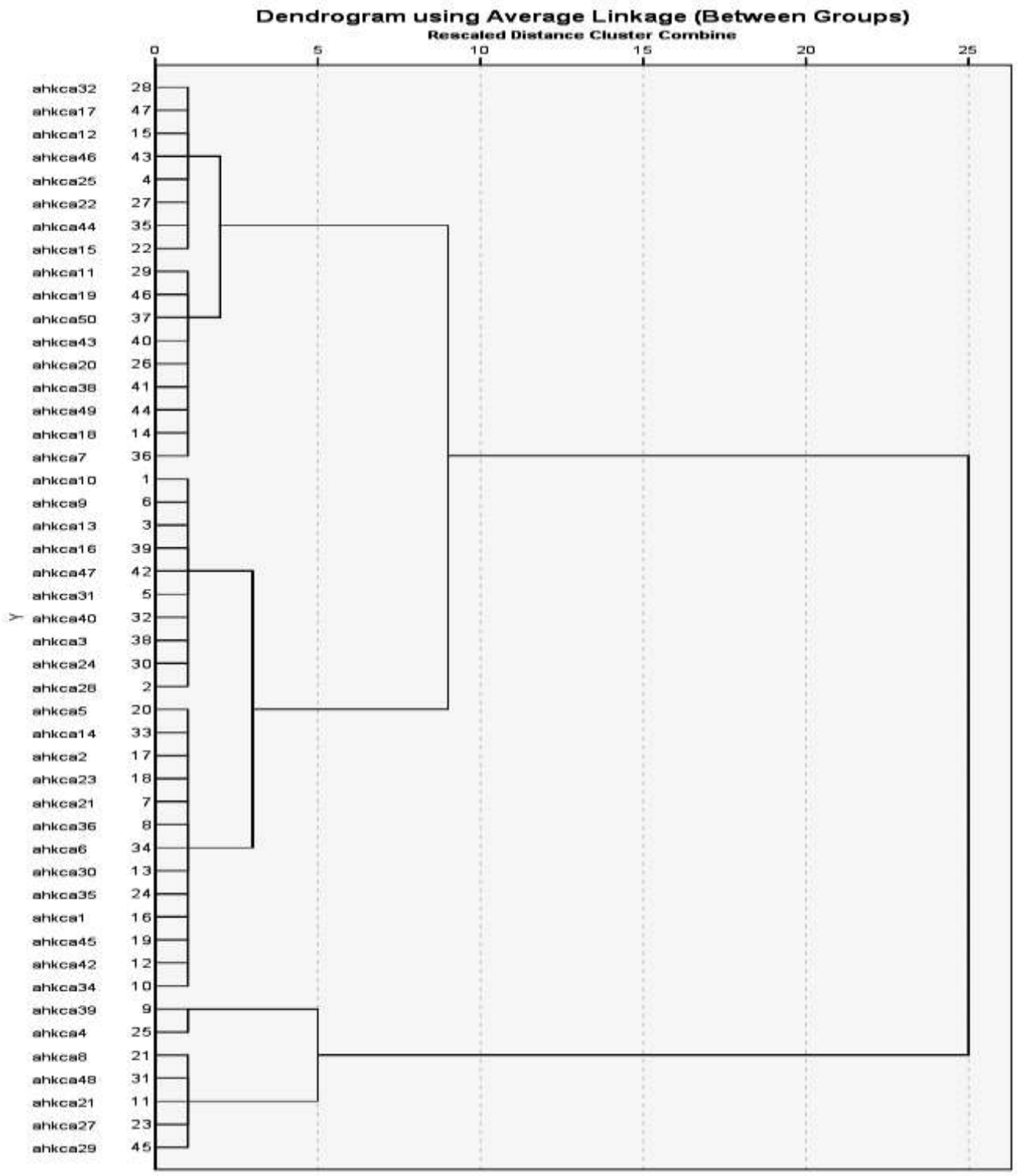

Figure 4. Direct hierarchic classification of hybrid genotypes of the subfamily ahkca (T79/501xUPA143).

defence of this plant was investigated. The assessment of seeds germination, the fourth and twelfth day after planting, showed that the germination rate of KHA family of hybrid genotypes is low compared to those of AHK and AHK3 families. Otherwise, the number of seeds from the pods KHA (27) family was lower than those of AHK (48)

Table 3. Phenotypic classes of disease scores of the family T79/501xUPA143 (AHK).

Subfamilies	Phenotypic classes of disease scores (% of hybrid genotypes)						
	0; 1	1; 1.5	1.5; 2	2; 3	3; 4	4; 4.5	4.5; 5
ahkca	6.38	6.38	4.26	31.91	31.91	19.15	0.00
ahkcb	5.26	7.89	13.16	26.32	31.58	13.16	2.63
Means	5.88	7.06	8.24	29.41	31.76	16.47	1.18

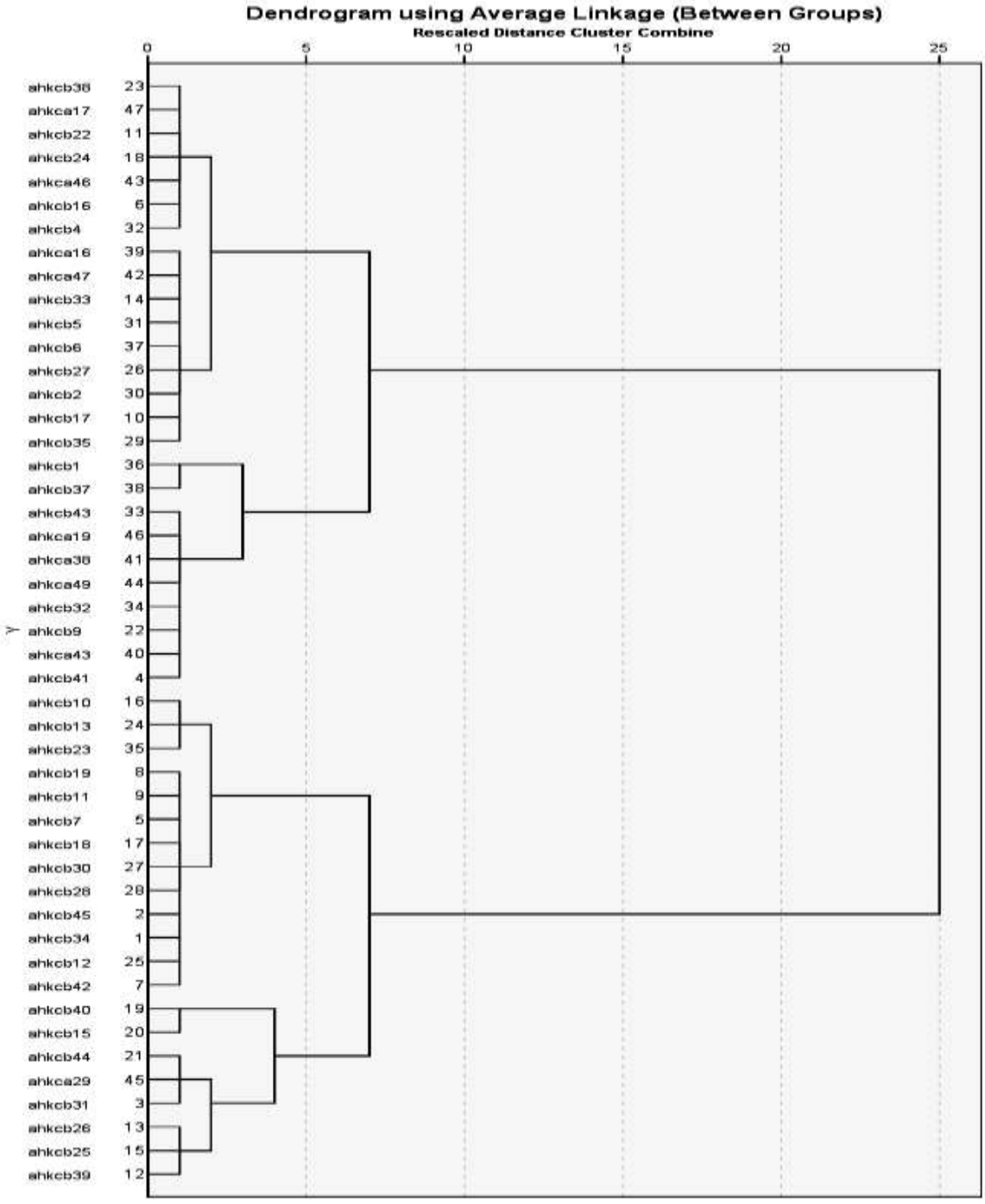

Figure 5. Direct hierarchic classification of hybrid genotypes of the subfamily ahkcb (T79/501xUPA143).

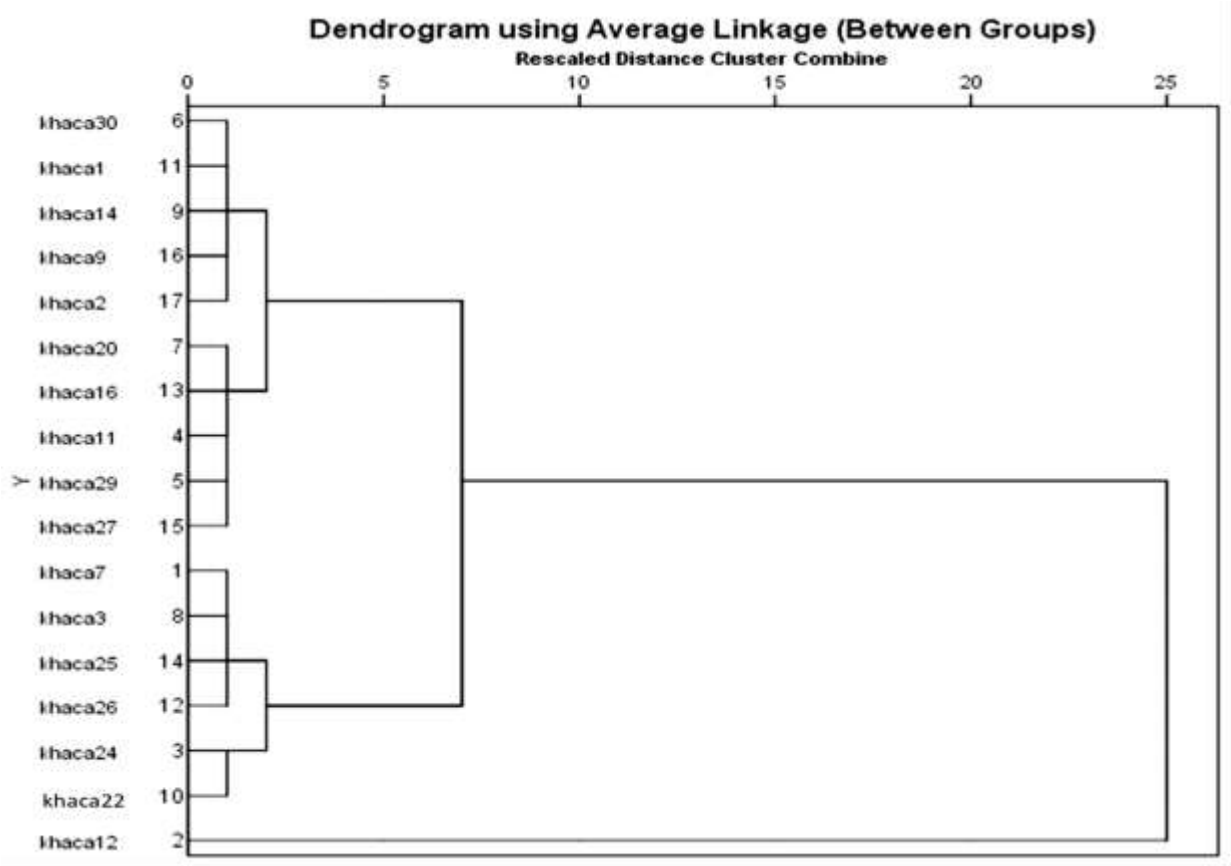

Figure 6. Direct hierarchic classification of hybrid genotypes of the subfamily khaca (SNK13xUPA143).

Table 4. Phenotypic classes of disease scores of the family SNK13xUPA143 (KHA).

Subfamilies	Phenotypic classes of disease scores (% of hybrid genotypes)						
	0; 1	1; 1.5	1.5; 2	2; 3	3; 4	4; 4.5	4.5; 5
khaca	5.88	0.00	0.00	29.41	35.29	17.65	11.76
khacb	11.76	17.65	17.65	5.88	23.53	23.53	23.53
Means	8.82	8.82	8.82	17.65	29.41	20.59	17.65

and AHK3 (46) families. These results are similar to those of some authors who reported that the germination rate and the number of seeds per pod are agronomic traits which guide preferably a crossover ratio to another (Liabeuf, 1967; Despréaux et al., 1989). Thus, considering only this stage of analysis based on these two characters, which are germination rate and number of seeds per pod, AHK and AHK3 families can be recommended to famers due to their high rate germination as compared to the KHA family.

Concerning the analysis of the sensitivity of hybrid genotypes of these three families, results showed the variability within and between families. This variability reflects the genetic heterogeneity of their offspring for the sensitivity character vis-a-vis to *P. megakarya*. Much research has, in fact, reported that the sensitivity of *T. cacao* to *P. megakarya* is a polygenic, non-cytoplasmic and nuclear character (Ndoumbe-Nkeng et al., 2001; Djocgoue et al., 2006; Nyadanu et al., 2013). This would mean that because of parental heterozygosity, no crossing would give rise to a very tolerant or completely susceptible offspring. When families are examined one by one, it was observed that the KHA family presented the highest percentage of sensitive hybrid genotypes compared to AHK and AHK3. These results are in conformity with those obtained by Nyasse et al. *(*2007) who reported that from the sensitivity of both parents (SNK13 and UPA143), SNK13 is moderately susceptible

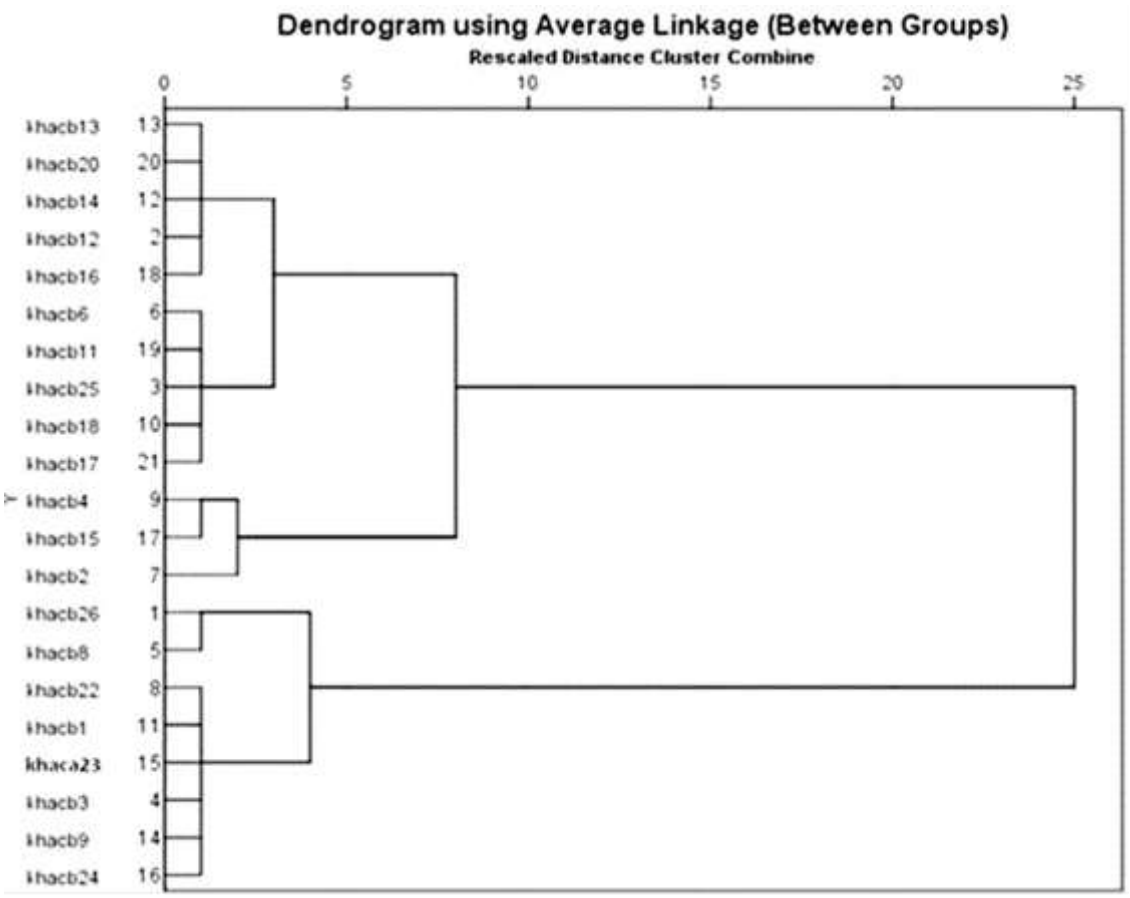

Figure 7. Direct hierarchic classification of hybrid genotypes of the subfamily khacb (SNK13xUPA143).

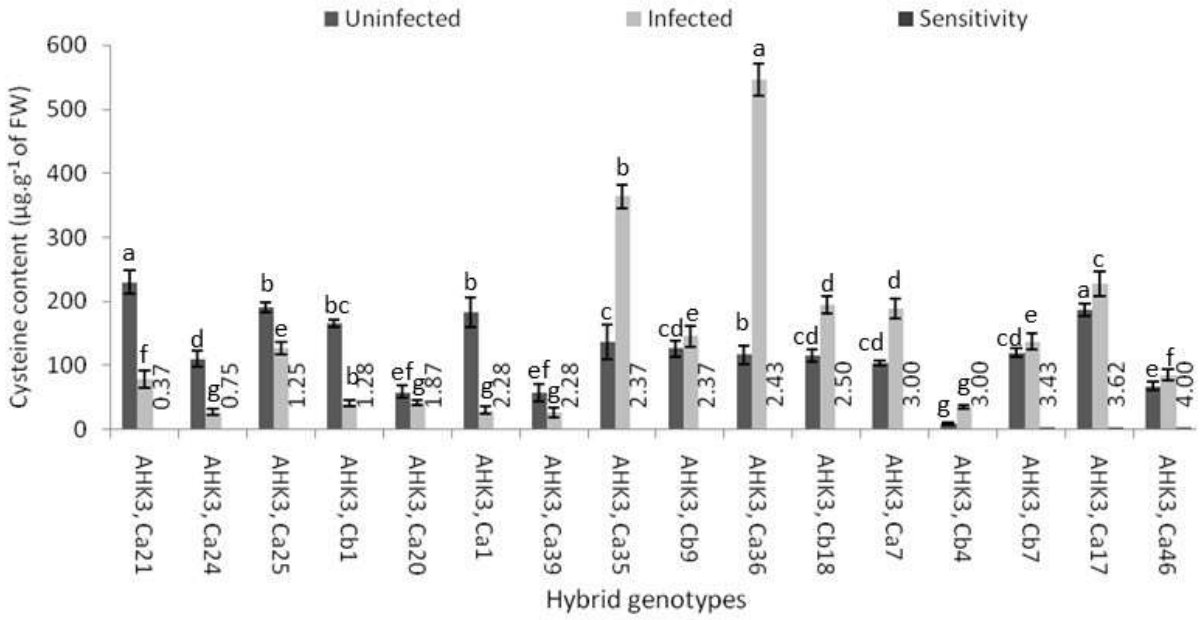

Figure 8a. Levels of free cysteine in leaves of some representatives of AHK3 family with their sensitivity. Values following by the same letter for a giving treatment are not significantly different (P< 0.05).

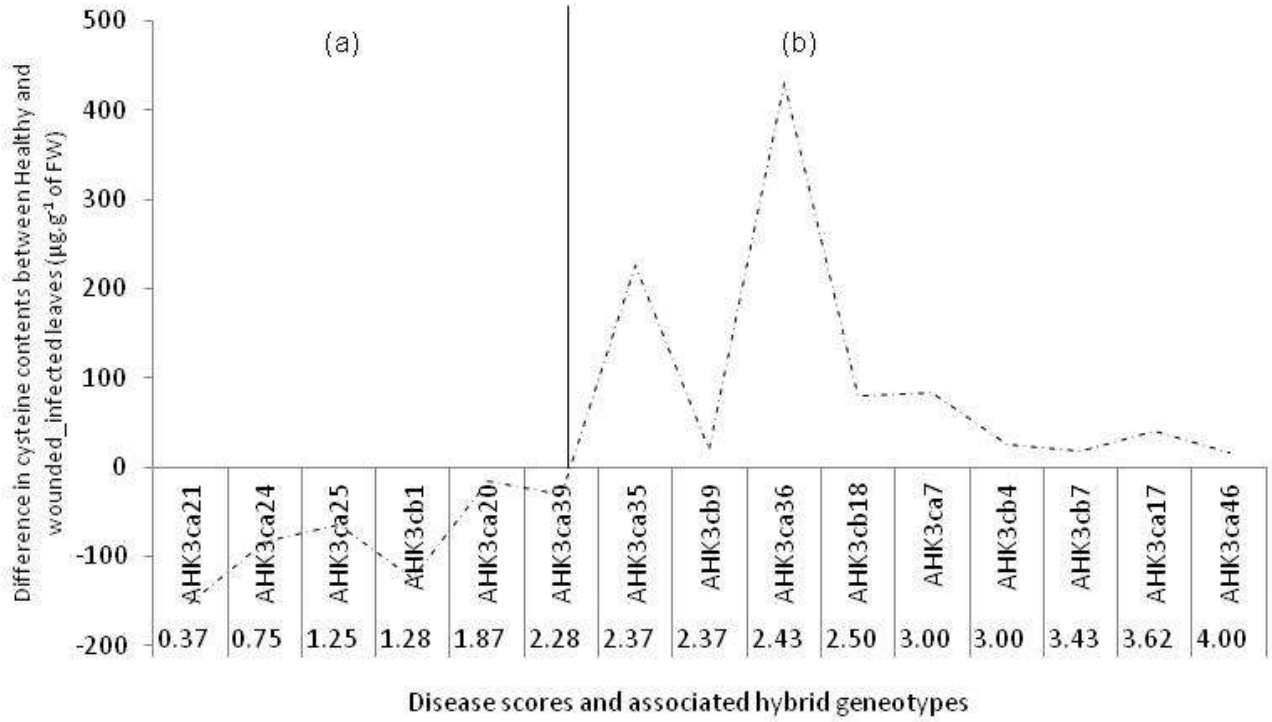

Figure 8b. Difference in cysteine content between healthy (H) and wounded-infected (W_I) leaves as function of disease scores of hybrid genotypes of AHK3 family. (a) = a group of tolerant hybrid genotypes (W_I – H) give negative value in cysteine content); (b) = a group of susceptible hybrid genotypes (W_I – H) give positive value in cysteine content).

and UPA143 has a high susceptibility to *P. megakarya*. It follows that the cross–of susceptible clones leads to a high proportion of susceptible individuals within the offspring. The crossover offspring resulted in three groups which consist of low, medium and high sensitivity. In the family T79/501xSNK13, the tolerant individuals' proportion is higher than the previous family, but lower than the AHK3 family. About the sensitivity of the parents, it has been reported that T79/501 is more tolerant than UPA143. Thus, the moderate sensitivity of SNK13 used as male parent contributes to the improvement of tolerance in the offspring (Nyasse et al., 2007).

The AHK3 family from the cross between UPA143xSNK64 is the one that resulted in the largest percentage of tolerant individuals compared to the previous two families. Certainly, UPA143 used as female parent is very sensitive but SNK64 used as male parent is classified as one of the most tolerant cacao clones to *P. megakarya*. Using SNK64 would be responsible for the high proportion of tolerant individuals observed in this cross. In other words, as SNK64 is the most tolerant clone of our study, we could say that this high rate is due to its ability as a male parent to transfer to the offspring the loci responsible for resistance to *P. megakarya*. According to some authors (Nyasse et al., 1995; Nyasse et al., 2002; Djocgoue et al., 2006; Tahi et al. 2006; Djocgoue et al., 2010), cocoa tolerance to *P. megakarya* is nuclear and not cytoplasmic. This explains that tolerance of cacao to *P. megakarya* is not related to mitochondria or chloroplasts and should therefore be influenced by the male parent. Our results are not in agreement with those of these researchers because during our studies we made direct crossings and not reciprocal crosses. On the other hand, observations in the nursery show that the development of necrosis is faster within the different families when it rains abundantly. These results could be explained by the fact that moisture gives favorable environmental conditions for the development of zoospores, responsible for the black pod disease. These results are similar to those obtained by Ngoh Dooh et al. (2015) who showed that the wide variation in rainfall during the two campaigns revealed an existing relationship between the fluctuations in rainfall and severity of the impact of black pods disease of cocoa.

Apart from the morphological aspect, the analysis of cysteine content in uninfected and infected leaves showed significant variations within representative groups of three hybrid families. In general, it appeared that, prior to infection (uninfected leaves), cysteine content varies from an individual to another. This soluble cysteine content appears to be associated with the level of sensitivity of hybrid genotypes. After infection, decrease in cysteine content was observed in tolerant hybrid

Table 5. Pearson Correlation using data of cysteine contents in healthy and wounded-infected leaves of hybrid genotypes from AHK3 family.

Parameter	Correlation staus	Disease scores of hybrids genotypes	Difference in cysteine content between healthy and wounded-infected leaves	Cysteine content in healthy leaves	Cysteine content in wounded-infected leaves
Disease scores of hybrids genotypes	Correlation	1			
	Sig.				
Difference in cysteine content between healthy and wounded-infected leaves	Correlation	0.670**	1		
	Sig.	0.009			
Cysteine content in healthy leaves	Correlation	-0.268	-0.479	1	
	Sig.	0.354	0.083		
Cysteine content in wounded-infected leaves	Correlation	0.472	0.636*	0.373	1
	Sig.	0.088	0.015	0.189	

* Correlation is significant at the 0.05 level (2-tailed); ** correlation is significant at the 0.01 level (2-tailed).

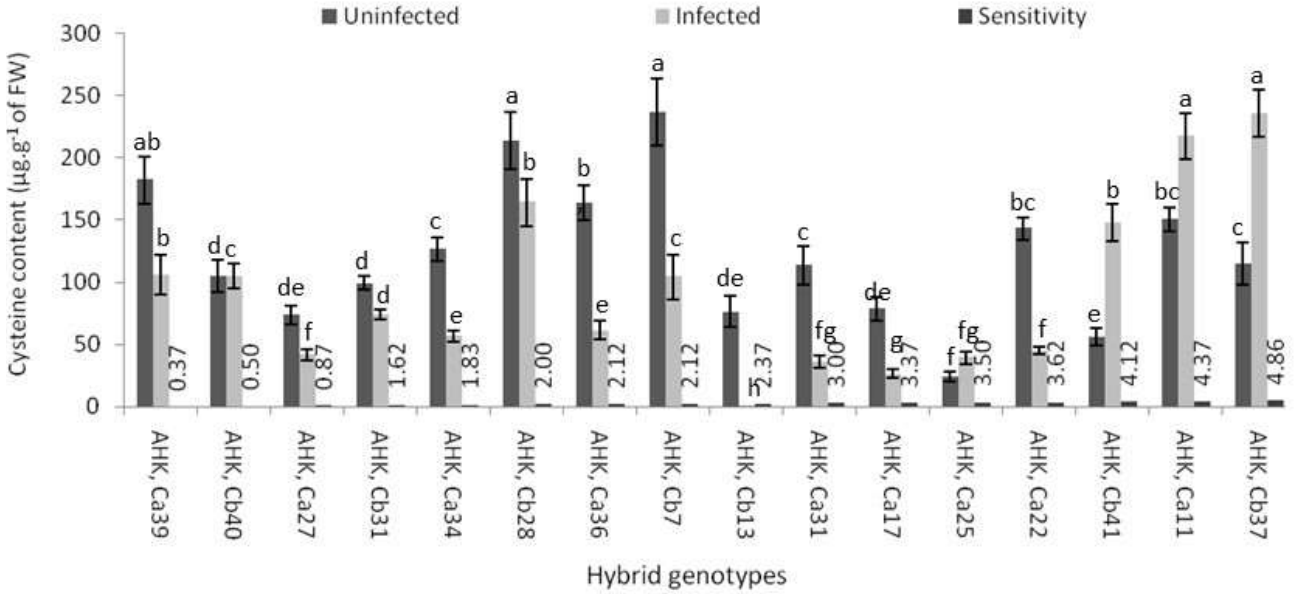

Figure 9a. Levels of free cysteine in leaves of some representatives of AHK family with their sensitivity. Values followed by the same letter for a given treatment are not significantly different (P<0.05).

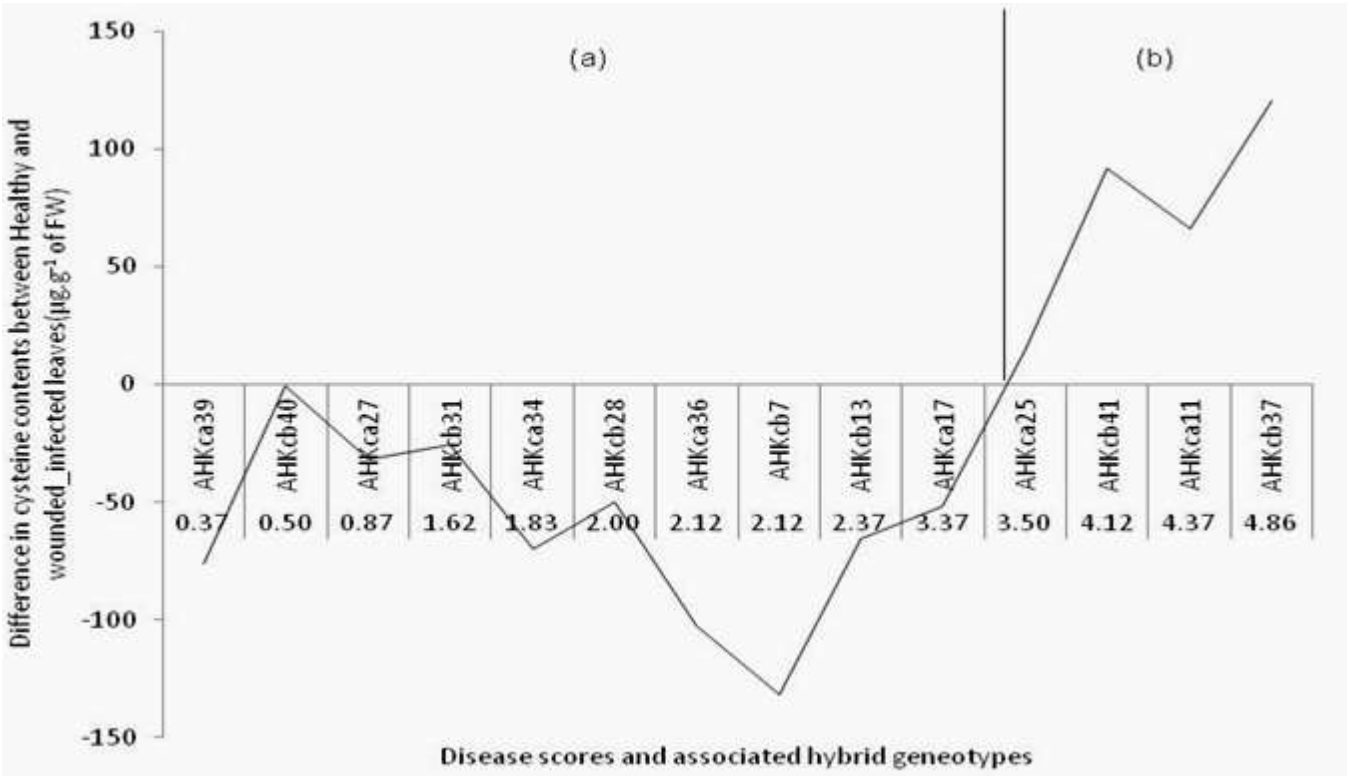

Figure 9b. Difference in cysteine content between healthy (H) and wounded-infected (W_I) leaves as function of disease scores of hybrid genotypes of AHK family: (a) = a group of tolerant hybrid genotypes (W_I – H) give negative value in cysteine content)and (b) = a group susceptible hybrid genotypes (W_I – H) give positive value in cysteine content).

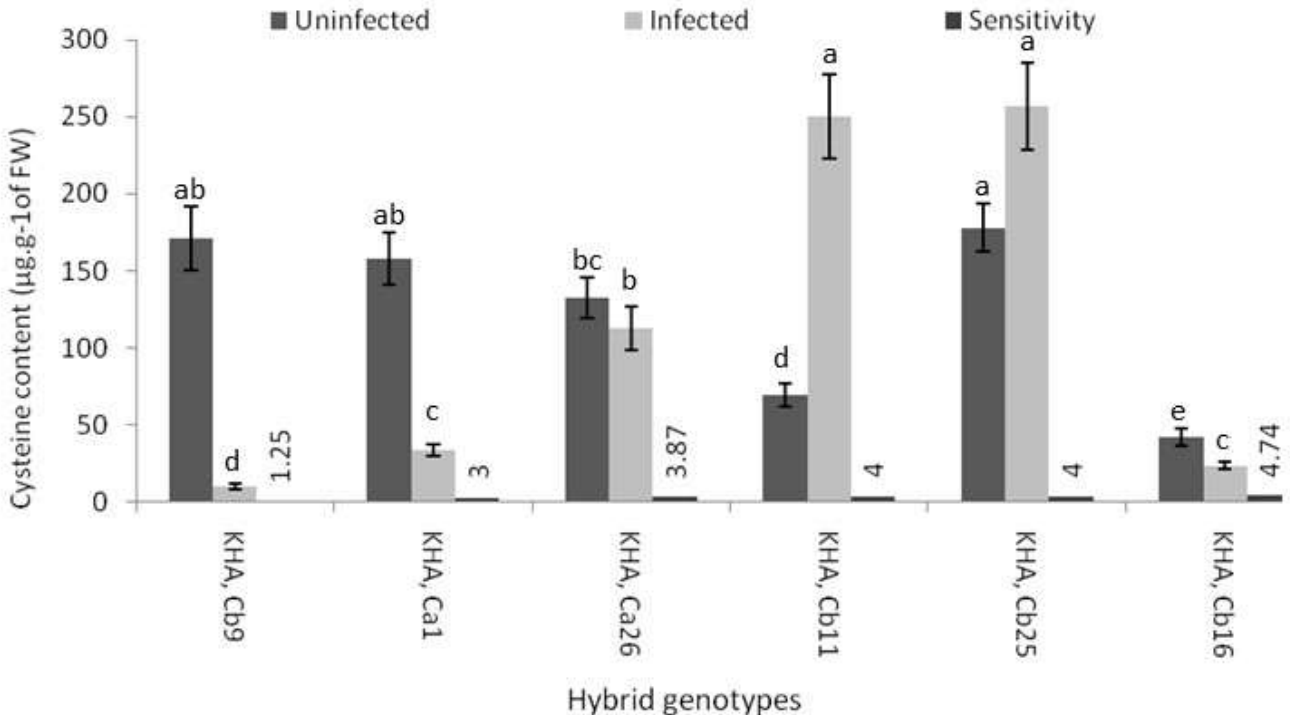

Figure 10. Levels of free cysteine in leaves of some representatives of KHA family with their Sensitivity. Values following by the same letter for a giving treatment are not significantly different (P< 0.05).

genotypes while in susceptible hybrid genotypes displayed a significant increase in cysteine content. Additionally, a positive and highly significant correlation was found between the reduction of cysteine content in infected leaves and disease scores of hybrid genotypes. These results are justified by the fact that in tolerant hybrid genotypes and in infection condition, cysteine is rapidly used by molecules to fight the pathogen. Reversely, in susceptible hybrid genotypes, cysteine synthesized may not be used during the infection. The hybrid genotypes KHACB16 KHA, CB11 and KHA, CB25 from KHA family susceptibility might be due to the fact that this hybrid genotype cannot mobilize cysteine for synthesis of sulfur containing molecules defense molecules during the pathogen attacks to ensure their defense. These observations may justify the high content of cysteine in the three hybrid genotypes after infection. Similar results were obtained from hybrid genotypes from AHK and AHK3 families. These results are in accord with those obtained by Cooper and Williams (2004) who reported that the synthesis of cysteine is triggered when the plant is exposed to parasitic attack. These results are also similar to those obtained by Borgen (2002) and Cerniauskaite (2010) who showed that certain metabolites containing sulfur such as glucosinolates are involved in defense against biotic attacks. In the similar approach, Saito (2000) showed that sulfur of organic sulfured metabolites is exclusively provided by cysteine. Thus, during the infection, dynamic of cysteine might have two phases in *T*. *cacao*: one phase synthesis accumulation, which leads to an increase of the cysteine content in the plant. In the second phase, cysteine is used (decrease of cysteine content) for the synthesis of sulfurous compounds involved in defense. There may exist therefore, an interdependence link between cysteine pools and the susceptibility to BPD of *T*. *cacao* hybrid genotypes when exposed to *P*. *megakarya* attack.

Conclusion

The susceptibility of hybrid genotypes of crosses SNK13xUPA143, T79/501xSNK13 and UPA143xSNK64 was evaluated and the involvement of cysteine in the defense of *T. cacao* against *P. megakarya* was analysed. The present investigation revealed that, crosses T79/501xSNK13 and UPA143xSNK64 gave the best germination rate and the highest number of seeds per pod. Similarly, both crosses generated the greatest number of tolerant hybrid genotypes. However, UPA143xSNK64 is the best of these three crosses.

The results of this study show that cysteine is mobilized during *P. megakarya* attack and quickly used in tolerant hybrid genotypes to fight against the pathogen. This biochemical analysis showed that cysteine pool could be used to discriminate tolerant from susceptible hybrid genotypes of *T*. *cacao*. Additionally, cysteine appeared to be a component of defense mechanism of T. cacao against *P*. *megakarya*. Tolerant hybrid genotypes from this study could be used by the famers to increase yield in their plantations. Moreover, the present finding could allow researchers to use cysteine in cocoa improvement program.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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