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Journal

Arthropod-Plant Interactions, 15(5)

ISSN

1872-8855

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Publication Date

2021-10-01

DOI

10.1007/s11829-021-09857-x

Arthropod-Plant Interactions (2021) 15:821–831
https://doi.org/10.1007/s11829-021-09857-x

ORIGINAL PAPER

Secondary metabolite effects of different cocoa genotypes on feeding preference of the mirid *Sahlbergella singularis* Hagl

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Received: 22 July 2020 / Accepted: 22 July 2021 / Published online: 31 July 2021

Abstract

Sahlbergella singularis is a major insect pest of cocoa in Cameroon. Conventional insecticides remain the most widely used option for mirid control, which unfortunately have adverse effects on the environment and human health. Improved methods of controlling this species, both environmentally friendly and inexpensive to farmers, are requirements. Varietal control based on the selection of resistant and/or tolerant genotypes can be an interesting approach. Nonetheless, the role of secondary metabolites (SMs) in cocoa defense against mirids is poorly documented; yet, these compounds are reported to be key elements in plant defense against herbivores. For this purpose, SMs of twelve cocoa genotypes were identified and quantified, as well as their impact on food preference by mirids. Food preference was assessed through microtests measuring cocoa attractiveness and antixenosis toward mirids. The results showed that cocoa genotypes were differently accepted as food by mirids, with a significant preference for hybrid IMC60 x SNK605 and a non-preference for T60/887. The ten other cocoa genotypes showed intermediate results. Five SMs classes: alkaloids, flavonoids, polyphenols, saponins, and tannins were identified. Their rates varied between cocoa genotypes: polyphenols > alkaloids > flavonoids > tannins, and saponins. Cocoa genotypes with high total phenolic contents were significantly preferred by *S. singularis* ($r_\alpha = 0.86$, $R^2 = 74.0\%$, $P < 0.001$), while those with low saponins contents were lowly accepted ($r_\alpha = -0.83$, $R^2 = 68.9\%$, $P < 0.015$), independently of the levels of other SMs. Given SMs high potential to affect mirid feeding behavior, analyzing cocoa SMs composition may help in early selection of resistant cocoa varieties against *S. singularis*.

Keywords *Theobroma cacao* · Biochemical analyses · Plant secondary metabolites · Attractiveness/antixenosis · Tolerant/resistant varieties · Insect-plant interactions

Introduction

Cultivated mainly by smallholders with low livelihood, cocoa *Theobroma cacao* L. (Malvales: Malvaceae) is an important source of income for rural communities of Central and West Africa (Wessel and Quist-Wessel 2015). However,

Handling Editor: Kerry Mauck.

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pest and disease damage to the crop significantly reduce cocoa yields by up to 40% (ICCO 2013).

In Cameroon, *Sahlbergella singularis* Haglund (Hemiptera: Miridae) is the most economically important insect pest of cocoa (Yede et al. 2012, 2016; Babin 2018; Mahob et al. 2020). This pest causes damage by injecting saliva through its stylet into the plant tissues, destroying them. Feeding lesions lead to desiccation of leaves, abortion of young fruits (also known as cherelles), and accumulation of cankers on pods and on the bark of trunk and branches (N'Guessan et al. 2008; Anikwe et al. 2009; Mahob et al. 2015, 2019, 2020). In some cases, *S. singularis* damage is associated with infections by opportunistic fungal species such as *Lasiodiplodia* spp., *Albonectria* spp., or *Fusarium* spp. (Adu-Acheampong et al. 2012, 2014; Anikwe and Otuonye 2015; Voula et al. 2018). These infections often cause cocoa tree dieback and death in a few months. In the absence of control measures, *S. singularis* damage on cocoa can cause annual production losses estimated between 10 and 100% (Yede et al. 2012; Babin 2018; Mahob et al. 2019).

To minimize *S. singularis* damage and associated cocoa losses, several control methods are recommended based on the bio-ecological data of the target pest. The most efficient method remains chemical control by targeted applications of synthetic insecticides of the neonicotinoid family, such as lambda-cyhalothrin and imidacloprid (Ayenoret al. 2007; Mahob et al. 2014). Other methods have been under study for decades—but with little use by farmers including (1) plant extracts and biological control with ants and entomopathogens (Mboussi et al. 2018; Bagny Beilhe et al. 2018a; Mahot et al. 2019); (2) shade management and plant diversification in cocoa plantations (Gidoin et al. 2014; Babin et al. 2010, 2018; Bagny Beilhe et al. 2018b); (3) pheromones for semiochemical control (Mahob et al. 2011; Sarfo 2013; Sarfo et al. 2018a,b; Mahot et al. 2020); and (4) resistant and/or tolerant cocoa genotypes for varietal control (Sounigo et al. 2003; N'Guessan et al. 2008, 2010; Mahob et al. 2019).

Although effective, chemical control has been expensive to farmers and has led to considerable side effects, such as a widespread insecticide resistance for targeted and non-targeted species, a decrease in the diversity and biomass of plants and animals, including useful beneficial arthropods like natural enemies and pollinators, and a general pollution of the environment leading to negative effects on human health (Geiger et al. 2010; Bagny Beilhe et al. 2018b; Humann-Guilleminot et al. 2019). Due to these risks, there is nowadays an increasing societal demand for organic cocoa farming that excludes the use of wide range of synthetic pesticides for cocoa pest and disease management (Babin 2018; Bagny Beilhe et al. 2018b).

One measure compatible with organic cocoa farming is the use of crop varieties that are resistant or tolerant to pests and diseases (Sharma and Rodomiro 2002; Cilas et al. 2018). Host plant selection, which can be based on various mechanisms, including plant detection and palatability before acceptance, is an important factor affecting herbivore fitness (Pickett et al. 1999; Bernal and Setamou 2003; Fuenzalida 2015; Wink 2016). Globally, there are on-going breeding programs for cocoa resistance to the main pests and diseases in Latin America, Asia, and Africa. Studies in Brazil for example gave good results for some major cocoa diseases, such as witches' broom disease caused by *Moniliophthora perniciosa* (Stahel) Aime & Phillips-Mora (Meinhardt et al. 2008). In West Africa, breeding for cocoa resistance to cocoa swollen shoot virus disease (CSSVD) notably is under investigation (Wessel and Quist-Wessel 2015; Ameyaw et al. 2014, 2017; Domfeh et al. 2016). Unfortunately, breeding for cocoa resistance to insect pests is lagging compared with other characteristics targeted in cocoa breeding. In the past 2 decades, several studies on cocoa resistance to mirids in Central and West Africa have shown that cocoa genotypes differ in their resistance and tolerance to mirid attacks with complex underlying mechanisms (Sounigo et al. 2003; Anikwe et al. 2009; N'Guessan et al. 2008, 2010; Mahob et al. 2019). Few studies measured mirid preference to different genotypes in laboratory feeding preference tests (Nguyen-Ban 1998; Badegana et al. 2004; Dibog et al. 2008). In this kind of choice tests, mechanisms involved in mirid response to cocoa genotypes may be of 2 types: attractiveness and antixenosis. A host plant's attractiveness is the set of traits (chemical and morphological) that are detected at a distance by the insect and that can promote attraction of the host plant to the insect for trophic and reproductive reasons (Morrison et al. 2019). Antixenosis or non-preference can be measured in choice or no-choice tests by the insect's probing behavior and the length of feeding time. Other plant defense mechanisms such as antibiosis and tolerance to cocoa mirids have been reported (Babin et al. 2005; N'Guessan et al. 2010; Sounigo et al. 2003, 2012). With antibiosis, the insect feeds on the host plant but cannot complete its development and does not reproduce due to adverse effects of the plant. Tolerance corresponds to the ability of the plant to withstand and/or recover from insect damage (Anikwe et al. 2009; N'Guessan et al. 2010).

It is well known that plant-derived products, such as secondary metabolites (SMs), play an important role in plant defense against a wide range of pathogens and herbivores by affecting plant acceptability as food (Hassan Adeyemi 2010; Fuenzalida 2015; Wink 2016; Yasri et al. 2018; Stenoien et al. 2019). SMs are known to be even more implicated in plant defense against herbivores such as mirids than morphological and phenotypic traits (Hassan Adeyemi 2010; Fuenzalida 2015; Erb and Kliebenstein 2020). Unfortunately, the implication of SMs in defense strategies of cocoa against mirid attacks is poorly documented. SMs belong to

three major groups: phenolic compounds (e.g., polyphenols, flavonoids, tannins), terpenoids (e.g., saponins), and alkaloids (Kabera et al. 2014; Fuenzalida 2015; Pagare et al. 2015; Kariñho-Betancourt 2018; Yasri et al. 2018). Numerous studies have reported a high capacity of many plants to synthesize a large variety of SMs, which have various ecological and biological activities on herbivores, such as anti-feeding, deterrence, repellence, antixenosis, attractance, and antibiosis. SMs are also involved in plant tolerance to herbivore damage (Krief 2003; Hilaly et al. 2004; Kabera et al. 2014; Pagare et al. 2015; Fuenzalida 2015; Yasri et al. 2018). In the present study, we focused on SMs that may be involved in cocoa attractiveness and antixenosis toward mirids (Cros et al. 1996). Indeed, understanding the direct role of these compounds in cocoa defense processes is necessary to support breeding programs for cocoa resistance to mirids. We hypothesized that the SM composition of the tested cocoa genotypes influences cocoa acceptance as food source by the mirid *S. singularis*.

Materials and methods

Study sites

The study was conducted between October 2018 and February 2019, jointly in the research station of the Agricultural Research Institute for Development (IRAD) of Nkoemvone (2°40′N and 11°20′E, 630 ma.s.l.), located in the South Region of Cameroon, and at the Food and Nutrition Research Center of the Institute for Medical Research and Studies of Medicinal Plants (IMPM) of Yaoundé (3°52′N and 11°31′E, 725 m above sea level), situated in the Center Region of Cameroon. Nkoemvone research station was chosen because it is within a major cocoa production basin in Cameroon, it is easily accessible, and it includes a laboratory of entomology and long-term untreated experimental plots of well-known cocoa genotypes (Mbondji Mbondji 2010; Mahob et al. 2019). In addition, practices of cocoa plot management, vegetation, climate, and soil characteristics of the study localities are well documented (Yede et al. 2012; Tadu et al. 2019).

Insect colony

Tests were performed using 4th to 5th larval instars of *S. singularis* because they are easy to handle compared with younger instars, which are more fragile, and adults that may fly to escape (Dibog et al. 2008; Voula et al. 2018; Mahob et al. 2019; Mahot et al. 2019). Mirids were collected from a colony maintained in an insectarium at IRAD, Nkoemvone, following methods of Babin et al. (2008) and Voula et al. (2018). The test insects were starved for 24 h before the start of the microtests.

Cocoa plant sources

Cocoa suckers measuring 12–25 cm were cut from trees of the 12 selected genotypes. Suckers were chosen, rather than branches, because they are preferred for feeding by mirids, as well as cocoa pods and twigs (Mariau 1999; Anikwe et al. 2009; Mahob et al. 2015). In addition, suckers are easy to handle for choice tests and usually pruned from trees as they are considered competitors to cocoa pod production (Mariau 1999). To avoid bias of a potential variability between trees of the same genotype, suckers were cut from the same cocoa trees (≈ 20 per genotype) and used for both microtests (attractiveness and antixenosis measures) and biochemical analyses of SMs.

Choice microtests

In the laboratory, suckers collected from cocoa trees were cut into 5-cm fragments. Only the fragments of diameter ≈ 1 cm and without any sign of pest attack were kept for the experiment (Nguyen-Ban 1998). Twelve cocoa genotypes, including eight hybrids: UPA143 × SNK64, T60/887 × SNK13, T79/501 × SNK13, IMC60 × SNK605, T79/501 × SNK64, IMC60 × SNK16, T60/887 × TIKO32, T79/501 × SNK16 and four clones: IMC60, T60/887, T79/501, PA35, were used for the experiments. These cocoa genotypes were chosen (1) to cover two groups of different genetic origin, i.e., Upper Amazon = IMC60, PA35, T60/887, T79/501 and UPA143 and local Trinitario = SNK and TIKO 32 (Dibog et al. 2008; Mbondji Mbondji 2010) and (2) on the basis of plant sucker availability during the study period. The method used was based on those of Nguyen-Ban (1998), Badegana et al. (2004), Dibog et al. (2008) and N'Guessan et al. (2010). Twelve plant-sucker fragments, one for each of the 12 tested cocoa genotypes, were randomly placed in a circular design, delimiting an observation arena of ≈ 15 cm diameter, on a laboratory bench previously covered with sterile paper towels to avoid any contamination (Fig. 1A) (N'Guessan et al. 2010). Three *S. singularis* nymphs were placed in the center of the arena, which afterward was enclosed in a sieve of 15 cm diameter to prevent the insects from escaping and to allow good aeration (Fig. 1B). After 24 h, mirid feeding lesions, which appear as dark green to black spots on sucker fragments, were counted. A total of 30 replicates of the experiment were conducted, meaning that a different section (sucker fragments) of each cocoa genotype was exposed 30 times to mirids (we used a new nymph batch for each replicate). The use of a control during the experiment was not necessary because of the random positioning

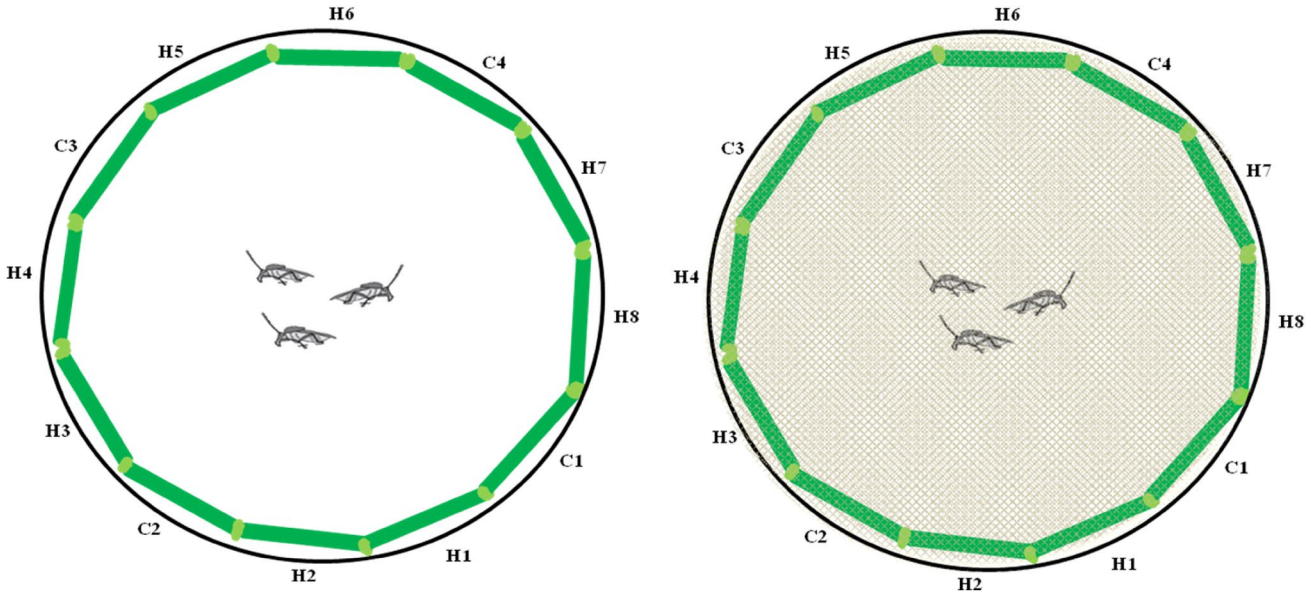

Fig. 1 Choice microtest design for the measurement of *S. singularis* feeding preference**,** with **A** open circular arena where green fresh cacao sucker fragments are placed with the codes of the different genotypes (H_i = code of i cacao hybrids tested, C_j = code of j tested clones) and 3 mirids in the center of the arena; **B** Arena enclosed using an aerated sieve

of cocoa suckers and mirids from one replicate to the other (Webster and Inayatullah 1988).

Biochemical analyses of cocoa genotypes

Preparation of plant extracts

Shortly after collection, cocoa suckers from each genotype were placed in sterile plastic bags (measuring 47 × 54 cm, one per genotype) during transport to the IMPM laboratory where they were rinsed with tap water and dried in a ventilated oven (Heraeus, Germany) at 60 °C for 72 h (Haque et al. 2003; Savithramma et al. 2011). The dried suckers were finely powdered using an electric grinder (Dhanani et al. 2013) and sieved to obtain powders with particle size less than 500 µm. The dry matter content of each genotype was determined using the gravimetric method (Anonymous 1997). Extracts were prepared by homogenizing 1 g of sucker powder in 30 ml of ethanol 80% for 1 h (Bassogog et al. 2020), using magnetic stirrer (Pyro-Multi-Magnestir, England). The mixture was then centrifuged (ROTOFIX 32 A, Japan) at 300 rpm during 30 min. The supernatant was recovered in Eppendorf tubes and stored at − 22 °C for subsequent manipulations.

SMs qualitative analyses

Aliquots of 2-ml extracts of plants were collected and used to identify the presence of SMs, namely polyphenols, flavonoids, tannins, alkaloids, and saponins, using methods described respectively by Djouahra (2012), Alzoreky and Nakahara (2003), Nwokonkwo (2009), Djeussi et al. (2013) and Nwokonkwo and Okeke (2014). Three replicates were randomly selected and pooled for each analysis.

SMs quantitative analyses

Total contents of each SM (viz: phenolics, flavonoids, tannins, alkaloids, and saponins) in each of the 12 cocoa genotypes were determined using standard protocols. Total phenolics content was measured using the Folin-Ciocalteu method with gallic acid as standard as described by Singleton et al. (1999). Total flavonoids content was determined through the method that uses aluminum chloride and sodium acetate with quercetin as standard described by Aiyegore and Okoh (2010). Total tannins were estimated using acidified vanillin as reported by Julkunen-Titto (1985). Total alkaloids content was evaluated using bipyridine by the method described by Singh et al. (2004) using quinin as standard. Total saponins content was determined by the method of Hiai et al. (1976) using saponin as standard. Analyses of each secondary metabolite were replicated 3 times. The content in each SM was expressed as mg of the corresponding standard per gram of dry weight (g dw).

Data analysis

The average number of mirid feeding lesions was recorded for each cocoa genotype per each replicate. Then, original data were log-transformed before analysis to correct for

inherent unequal variances of count data. Finally, cocoa genotypes were compared for the mean number of feeding lesions using an univariate analysis of variance (ANOVA), followed by the Tukey's HSD post hoc test for pairwise comparisons of the multiple means of feeding lesions of 12 cocoa genotypes. The rates of the different SMs (three per genotype) were ranked for each genotype, and then the median values (or mean ranks) were compared between the cocoa genotypes using the H-test of Kruskal–Wallis, without data transformation, which was not needed. A Bonferroni post hoc test was performed for pairwise comparisons of the medians. In addition, the relationship between the number of feeding lesions and the rates of the three major groups of SMs (i.e., total phenolic compounds, alkaloids, and saponins) per each cocoa genotype sample was estimated using Spearman's correlation analysis. Furthermore, the degree of similarity of the different genotypes for their SMs was determined using a multivariate analysis (Cluster analysis) by considering cocoa genotypes as line individuals and SMs as column individuals. All statistical analyses were performed with STATISTICA (version 10) software and the differences were appreciated at the 5% confidence level.

Results

Assessment of *S. singularis* preference to different cocoa genotypes

The mean number of feeding lesions varied significantly between the different cocoa genotypes. The ANOVA revealed five homogenous groups for the numbers (means ± ES) of *S. singularis* feeding lesions, suggesting a significant preference ($F_{(11,348)} = 303.1$, $P < 0.001$) for IMC60 × SNK605 and a significant non-preference for T60/887 (Table 1). The other cocoa genotypes tested showed intermediate and comparable results (Table 1).

Table 1 Mean (± SE) number of *S. singularis* feeding lesions on sucker fragments of the twelve cacao genotypes tested in choice microtests

Cacao genotypes	Number of mirid feeding lesions
T60/887	2.70 ± 1.15^{a}
T79/501 × SNK13	3.10 ± 0.70^{ab}
T79/501 × SNK16	3.60 ± 1.02^{ab}
PA35	4.10 ± 1.04^{abc}
IMC60	5.00 ± 1.23^{abc}
T79/501 × SNK64	6.10 ± 1.14^{abc}
IMC60 × SNK16	7.50 ± 1.42^{abc}
T79/501	7.90 ± 1.43^{abc}
T60/887 × SNK13	8.20 ± 1.40^{abc}
UPA143 × SNK64	8.20 ± 1.02^{abc}
T60/887 × TIKO32	8.80 ± 1.53^{bc}
IMC60 × SNK605	15.10 ± 1.80^{d}

Values expressed are mean ± standard deviation. Values with different letters are significantly different at $P < 0.05$

Assessment of the SMs in cocoa genotypes

Qualitative identification of the SMs

The results of the qualitative analyses showed that five SM classes: polyphenols, tannins, flavonoids, alkaloids, and saponins were present in the 12 tested cocoa genotypes.

Quantitative comparison of the SMs

The rates of SMs varied among genotypes. Table 2 showed that total phenolic contents were significantly higher than other SMs in all the genotypes. Median values of SMs were also significantly different between the genotypes; three compounds: tannins, flavonoids, and saponins, showed the lowest average levels in the whole genotypes. The highest total phenolic content was observed in IMC60 × SNK605 (59.7 ± 0.30 mg GAE/g dw), and the lowest in T60/887 (49.6 ± 0.13 mg GAE/g dw). Regarding tannins, the lowest value was obtained in T60/887 (0.3 ± 0.03 mg GAE/g dw) and the highest in T79/501 × SNK64 (1.3 ± 0.05 mg GAE/g dw). The lowest flavonoids value was obtained in IMC60 (0.8 ± 0.02 QE/g dw) and the highest in T79/501 × SNK64 (2.7 ± 0.02 QE/g dw). For alkaloids, the lowest value was recorded in IMC60 (18.8 ± 0.01QuiE/g dw) and the highest value in IMC60 × SNK16 (24.6 ± 0.2 QuiE/g dw). For saponins, the lowest value was observed in T60/887 × TIKO32 (0.4 ± 0.01 SE/g dw) and IMC60 × SNK605 (0.4 ± 0.04 SE/g dw) and the highest in T60/887 (1.8 ± 0.01 SE/g dw), T79/501 × SNK16 (1.8 ± 0.01 SE/g dw), and T79/501 × SNK13 (1.8 ± 0.04 SE/g dw) (Table 2).

Correlation between SM content and number of feeding lesions

Results showed that the number of mirid feeding lesions on sucker fragments was influenced by the quantity of their SMs. The number of mirid feeding lesions on cocoa sucker fragments was positively correlated with the total of phenolic compounds ($r_{\alpha} = 0.86$, $R^2 = 74.0$, $P < 0.001$), negatively correlated with the total of saponins ($r_{\alpha} = -0.83$, $R^2 = 68.9\%$, $P < 0.015$), and not correlated with total alkaloids ($r_{\alpha} = 0.23$, $R^2 = 5.3$, $P = 0.58$).

Table 2 Secondary metabolic levels of different cacao genotypes

Genotypes	Polyphenols (mg GAE/g dw)	Tannins (mg GAE/g dw)	Flavonoids (mg QE/g dw)	Alkaloids (mg QuiE/g dw)	Saponins (mg SE/g dw)
UPA143 × SNK64	56.5 ± 0.22^{d}	1.2 ± 0.21^{bc}	2.5 ± 0.08^{cd}	23.8 ± 0.08^{cd}	0.5 ± 0.02^{ab}
T60/887 × SNK13	57.8 ± 0.15^{f}	0.4 ± 0.27^{a}	2.0 ± 0.03^{abc}	21.9 ± 0.01^{ef}	$0,5 \pm 0.02^{b}$
T79/501 × SNK13	50.8 ± 0.37^{a}	1.0 ± 0.25^{bc}	2.0 ± 0.04^{abcd}	23.6 ± 0.04^{bcd}	1.8 ± 0.04^{d}
IMC60 × SNK605	59.7 ± 0.30^{g}	0.7 ± 0.07^{abc}	1.9 ± 0.03^{abc}	23.2 ± 0.01^{abd}	0.4 ± 0.04^{a}
T79/501 × SNK64	54.1 ± 0.30^{c}	1.3 ± 0.05^{c}	2.7 ± 0.02^{d}	23.8 ± 0.13^{c}	0.8 ± 0.04^{g}
IMC60 × SNK16	54.2 ± 0.44^{c}	0.8 ± 0.07^{abc}	2.3 ± 0.04^{bcd}	24.6 ± 0.19^{g}	0.5 ± 0.01^{b}
T60/887 × TIKO32	57.6 ± 0.17^{e}	$0.7 \pm 0,01^{abc}$	1.7 ± 0.06^{af}	23.1 ± 0.21^{ab}	0.4 ± 0.01^{a}
T79/501 × SNK16	50.8 ± 0.23^{a}	0.8 ± 0.13^{abc}	$2.1 \pm 0,04^{abcd}$	24.1 ± 0.01^{cg}	1.8 ± 0.01^{d}
IMC60	53.4 ± 0.20^{bc}	0.6 ± 0.13^{ab}	0.8 ± 0.02^{e}	18.8 ± 0.01^{h}	0.7 ± 0.04^{c}
T60/887	49.6 ± 0.13^{a}	0.3 ± 0.03^{a}	1.1 ± 0.01^{ef}	21.5 ± 0.01^{e}	1.8 ± 0.01^{e}
T79/501	55.7 ± 0.33^{d}	0.6 ± 0.23^{ab}	1.7 ± 0.01^{ab}	22.4 ± 0.03^{af}	0.7 ± 0.01^{c}
PA35	52.2 ± 0.15^{b}	0.7 ± 0.13^{abc}	$1,2 \pm 0.15^{cf}$	22.7 ± 0.09^{ab}	1.7 ± 0.02^{d}
Statistics	$H = 31.8$, $P < 0.0001$	$H = 47.3$, $P < 0.0001$	$H = 39.9$, $P < 0.0001$	$H = 27.8$, $P < 0.0001$	$H = 44.8$, $P < 0.0001$

Values expressed are mean ± standard deviation. Values within the same column with different letters are significantly different at $P < 0.05$

Estimation of the degree of SMs similarity between the cocoa genotypes

Cluster analysis divided the 12 cocoa genotypes into two homogeneous subgroups (A and B), within which there were also close similarity of SMs by pairs due to the different genetic origins of the cocoa genotypes (Fig. 2).

Discussion

The goal of the present study was to improve our understanding of the direct role of SMs in *S. singularis* preference for cocoa genotypes as food source. The preference was measured through the number of feeding lesions on sucker fragments. Biochemical analyses revealed the presence of polyphenols, alkaloids, tannins, flavonoids, and saponins in the selected cocoa genotypes, as previously

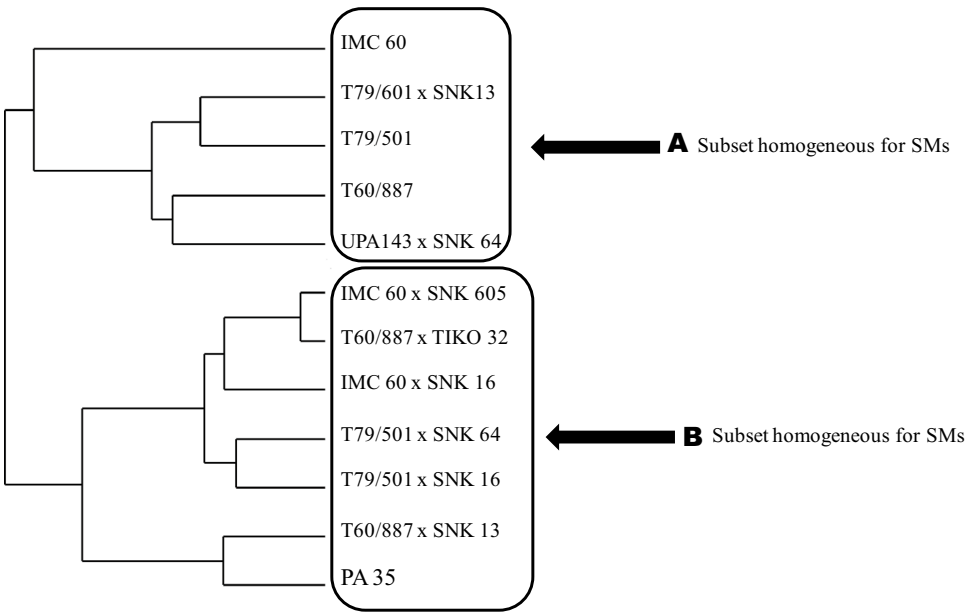

Fig. 2 Similarity of the SMs between the studied cacao genotypes, according to the Cluster analysis. **A** and **B** Homogeneous subsets in term of SMs composition

reported on different cocoa organs (beans, leaves, and bark) by Nwokonkwo and Okeke (2014) in Nigeria, Subhashini et al. (2010) in India and Hii et al. (2009) in Asia. Our study shows that *S. singularis* significantly prefers sucker fragments from IMC60 × SNK605 cocoa genotype and the species is repelled by T60/887 genotype, while the 10 other cocoa genotypes showed intermediate results. Our results thus support the hypothesis that SM heterogeneity (in terms of SM contents) of plants strongly affects the feeding ecology of mirids, and confirms the observation that SMs play a critical role in plant defense against herbivores (Mithöfer and Boland 2012). The differences in *S. singularis* food preference in the current study appeared to be associated with the high and low cocoa SM contents of phenolic compounds and saponins respectively, independently of other compounds. Thus, antixenosis was clearly recorded in genotype T60/887, which showed very few lesions of *S. singularis* feeding, while a high preference was observed for genotype IMC60 × SNK605 based on the highest number of *S. singularis* lesions on their sucker fragments (Nguyen-Ban 1998). It is known that, for many insects including mirids, an avoidance of inappropriate host plant depends on initial attempts at feeding or colonization, which involves nutritional quality, digestibility, and/or detection of compounds potentially or really toxic to herbivores, as well as possibly other food aspects (Pickett et al. 1999; Bernal and Sétamou 2003; Fuenzalida 2015; Wink 2016). Our findings support those of other studies showing that attractiveness and/or antixenosis of plants to herbivores often depend on the presence of specific phytochemicals (Kariñho-Betancourt 2018; Yasri et al. 2018); however, the precise mode of action of cocoa attractiveness to mirids as well as the one used by this pest to select host plants (preference or antixenosis) remains to be elucidated. Such knowledge improvement of mirid feeding on cocoa can be potentially incorporated into *S. singularis* control strategies via antixenosis mechanism for example, which could potentially lead to more efficient breeding programs and substantial reduction in synthetic insecticide use for mirid control in cocoa plantations. Moreover, the reduction in the use of synthetic insecticides in cocoa plantations could substantially improve the conservation of cocoa flower pollinators, which are essential for improving cocoa pod yield (Toledo-Hernandez et al. 2017), and lead in long term to increase production of organic cocoa to meet increasing world-wide demand for organic cocoa products (Babin 2018; Cilas et al. 2018).

In our investigations, mirid feeding lesions strongly varied among cocoa genotypes, as reported by Badegana et al. (2004) and Dibog et al. (2008) in Cameroon and by N'Guessan et al. (2008, 2010) in Ivory Coast. However, the specific levels of feeding lesions on the genotypes in these studies differed from those obtained in our study. For instance, feeding lesions values were estimated between 5.39 for clone ICS100 and 6.18 for clone NA33 (Badegana et al. 2004), 2.44 for clone IMC60 and 4.96 for clone BE10 (Dibog et al. 2008) in Cameroon versus 2.00 for clone Playa Alta2 and 10.28 for clone ICF372 in Ivory Coast (N'Guessan et al. 2008, 2010). These numerical differences between the studies could be explained by the heterogeneity of the different experimental and environmental conditions as well as mirids and cocoa genotypes used (Thomas et al. 2012). Cocoa genotypes tested in our study were mainly made-up of cocoa hybrids (8 of the 12 specimens tested) while those used by the above-mentioned authors only focused on clones. Sounigo et al. (2003, 2012) reported that cocoa hybrids are significantly more susceptible to mirid attacks compared with cocoa clones; this could justify, among other factors, the relatively higher values of feeding lesions observed in our investigations compared with those obtained by the other authors.

Moreover, studies reported from Cameroon, Nigeria, and Ivory Coast (see above) correlated the differences in mirid feeding lesions to differential attractiveness and/or resistance/tolerance of cocoa genotypes to *S. singularis* attacks. Our study adds evidence that the observed differences in the levels of mirid feeding lesions depend on SM composition, thus complementing earlier genetic data (Sounigo et al. 2003; N'Guessan et al. 2008, 2010; Cilas et al. 2018). Regardless of other compounds, *S. singularis* responds negatively to cocoa genotypes with high saponins contents, resulting to fewer feeding lesions, characterizing antixenosis. By contrast, high level of phenolic compounds correlates positively with more feeding lesions, characterizing a feeding preference. Thus, derivatives of phenolic compounds (polyphenols, flavonoids, and tannins) appeared to be involved in food preference by *S. singularis*, while saponins could be involved in avoidance. Phenolic compounds especially flavonoids are known to be attractive and preferred food to herbivores such as mirids (Cros et al. 1996; Kariñho-Betancourt 2018). However, the anti-feeding and toxic effects of tannins and polyphenols on herbivores such as mirids have not been demonstrated in this work because all the cocoa genotypes tested contained high concentrations of polyphenols compared with the other compounds. This result could be associated with a phenomenon of selective food preference by mirids toward these compounds in cocoa genotypes driven by adaptation by the herbivore to plant nutrients, including plant toxins (Tabashnik et al. 1998). Saponins, which are derived from the terpene's group, showed antixenosis activity toward mirids in our study, thus suggesting toxicity and/or anti-feeding and anti-digestive activities as demonstrated for other members of Miridae family (Bennett and Wallsgrove 1994; Rafińska et al. 2017; Kariñho-Betancourt 2018; Yasri et al. 2018). Although the influence of saponins and phenolic compounds on the mirid food choice has been generally established, this

study showed that about 25–31% of the *S. singularis* feeding choice does not depend on both compounds. Therefore, it is likely other mechanisms are involved in cocoa defense against mirids such as defensive proteins like Trypsin Proteinase Inhibitors, phenotypic/physical traits, etc. (Tamayo et al. 2000; Kessler 2006). These mechanisms need to be explored to enhance our knowledge of mirid feeding ecology.

In addition to saponins and phenolic compounds, our study found that alkaloids do not appear to affect mirid feeding ecology; however, their direct effect on the attractiveness/antixenosis to *S. singularis* has not been clearly demonstrated. The low value of R^2 (less than 6%) and the extreme variation of their levels in cocoa genotypes leads to the hypothesis that alkaloids could play a role in other mechanisms of cocoa defense against mirids such as deterrence or resistance/tolerance. Additional studies are therefore necessary to elucidate this assertion.

The results of our experiments clearly revealed that SMs of the cocoa genotypes influence feeding behavior of *S. singularis*. Cocoa genotypes with high concentration of phenolic compounds and low concentrations of saponins were preferred as food by *S. singularis*. However, the alkaloids were not clearly involved in the feeding ecology of *S. singularis* due to irregular fluctuation of their concentrations in the host plants, and the very low linear correlation observed between rates of these compounds and the attractiveness/antixenosis of *S. singularis*. That phenolic compounds and saponins contents of cocoa genotypes evidently play a decisive role in the *S. singularis* feeding ecology, strongly suggests that SMs should be incorporated into cocoa breeding programs for the integrated control of *S. singularis* in West and Central Africa.

Acknowledgements This study was funded by the public investment funds of Cameroon through the presidential allowance to the modernization of research. We thank Food and Nutrition Research Center of the Institute of Medical Research and Study of Medicinal Plants for logistic and lab products/reagents. We are also grateful to the Research Station of IRAD-Nkoemvone, especially Mr Damien EYENET and Laurent BALEBA, Technician and Station Manager respectively, for their collaboration during the field data collection phase related to the genetic origins of the tested cocoa genotypes. We also thank Dr Njua Clarisse, Senior Lecturer at the Faculty of Science of the University of Yaoundé I, and Dr Ayuk Tambe Bertrand, Lecturer at the Faculty of Health Sciences of the University of Buea, for their contributions in the English proofreading.

Author contributions RJM, CFBB, and RH: Conceived and planned the study; RJM, IMN, RFD, CHM, CBB, FEE, PBNE, and DT: Designed and performed the experiments in both field and laboratory; RJM, CFBB, and RH: Discussed on the statistical methods and performed statistical analysis; RJM, RFD, CFBB, RH, and RB: Wrote the manuscript.

Declarations

Conflict of interest This work does not present any conflict of interest.

Consent for publication All authors agree that the manuscript be published for the benefit of the scientific community and/or sympathizers and other actors in the cocoa sector.

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