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Effect of genotype of cocoa (*Theobroma cacao*) on attractiveness to the mirid *Sahlbergella singularis* (Hemiptera: Miridae) in the laboratory

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Abstract

BACKGROUND: Mirids are a major constraint to cocoa growing in Africa. Cocoa breeding for tolerance/resistance could be effective in an integrated pest management system. Attractiveness is one aspect of tolerance/resistance, and decreasing the attractiveness of cocoa trees should be a good way of reducing damage.

RESULTS: Small-scale laboratory tests were carried out in Cameroon to assess differences in the attractiveness to mirids of eleven cocoa genotypes. The genotypes were ranked according to their attractiveness score and a distance from a mean value. An analysis of variance was performed and revealed significant differences between cocoa genotypes ($F = 3.15$, $P < 0.001$). The cocoa genotype groupings revealed three major categories, with BE10 and SNK413 proving to be the most attractive. In contrast, five genotypes, IMC60, the Catongo Trinitario genotypes, Playa Alta2 (from Venezuela), SIC5 and SNK614, proved to be less attractive than the mean. Four genotypes (PA107, SNK619, UPA134 and T60/887) displayed similar attractiveness to the mean.

CONCLUSION: The circular microtest offered the advantage of not needing a reference cocoa genotype. The least attractive clones, such as IMC60, were also the most tolerant in the field. Comparisons with the results of other studies are proposed.

Keywords: circular combinations; microtest; insect feeding points; cocoa mirids

1 INTRODUCTION

Originally cultivated in Latin America, cocoa trees are nowadays widely grown in the tropics.[1] For some developing countries, cocoa is among the crops that provide the greatest source of foreign currency.[2] An important approach for research activities is the evaluation of cocoa genotypes,[3,4] mostly for improving cocoa tree productivity.[5–7] More recently, research on cocoa has become increasingly focused on the control of diseases[8,9] and insect pests[10–16] which can substantially reduce cocoa production. Cocoa mirids, *Sahlbergella singularis* Hagl. and *Distantiella theobroma* Dist. (Heteroptera: Miridae), are the major pests of cocoa in West Africa, causing 30–40% losses in production.[17,18] Their feeding points cause the drying up of young branches and the formation of cankers which allow entry of parasitic fungi into the tree. In order to limit this pest's damage, an integrated pest management (IPM) programme was considered.[19] In such an IPM, the improvement of cocoa genotypes for resistance/tolerance is a key factor. As with most perennial crops, selection of cocoa trees for any genotype character, such as resistance or tolerance to mirids, is time consuming and costly.[20] The microtest technique described by Nguyen-Ban[21] enables an early selection of cocoa genotypes, taking into account their attractiveness towards mirids, which could help to shorten the length of study periods for cocoa resistance to these pests. The goal of this paper is to evaluate results of triangular microtests conducted in Cameroon according to the method proposed by Nguyen-Ban.[21]

2 MATERIALS AND METHODS

2.1 Materials

Eleven cocoa genotypes were used for the microtests: four Upper Amazon Forasteros, Pa 107, T 60/887, IMC 60, UPA 134; three Lower Amazon Forasteros, SIC 5, BE 10, CATONGO; and four Trinitarios, SNK 413, SNK 614, SNK 619, PLAYA ALTA2.

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Table 1. Types of international cocoa genotypes, genotypes tested and triangular combinations made for microtests: Up. Am. = Upper Amazon; Loc. Trin. = Local Trinitario; L. Am. F. = Lower Amazon Forastero; Trin. u. p. = Trinitario unknown parents. Genotypes with the same combination experimental number were tested together

		Experimental combinations										
Type	Cocoa genotypes	1	2	3	4	5	6	7	8	9	10	11
Up. Am.	PA107	X	X	X								
Loc. Trin.	SNK413	X	X									X
L. Am. F.	SIC5		X	X	X							
L. Am. F.	BE10			X	X	X						
Up. Am.	T60/887				X	X	X					
L. Am. F.	CATONGO					X	X	X				
Loc. Trin.	SNK614						X	X	X			
Up. Am.	IMC60							X	X	X		
Trin. u. p.	Playa Aita2								X	X	X	
Loc. Trin.	SNK619									X	X	X
Up. Am.	UPA134	X									X	X

The combinations of genotypes in the experimental design are shown in Table 1.

The tests were performed with fourth- and fifth-instar nymphs of *S. singularis*, the most economically important and abundant mirid species in Cameroon.

2.2 Methods

The method is adapted from Nguyen-Ban.[21] Three sections of young branches, 50 mm in length and with similar diameters, were arranged in a triangle and stapled together in a 9 cm diameter petri dish. Mirid nymphs were captured from the field early in the morning, and specimens were kept in a box with young cocoa branches during the day. From the evening of the same day, the nymphs were kept without food through the following night. The next morning, one mirid nymph was introduced into each petri dish, and, 24 h later, mirid feeding points were counted on each branch.

Each genotype was tested 3 times in a circular-design test (Table 1), and the use of a systematic control clone was therefore not necessary. This type of partial factorial design has already been used in other studies on relationships between insects and varieties.[22] A triangular microtest reduces the choice of the insect to three possibilities and simplifies the interpretation.

For each new combination, two of the genotypes within the previous combination were kept, and the third one was replaced. The genotypes were ranked in each combination, from 1 (the highest number of feeding points) to 3 (the lowest). For the final score, the least attractive genotypes should score 9 (from three combinations), and the most attractive should score 3. The score would therefore rank the performance of a genotype compared with the four others.

2.3 Statistical analysis

Cocoa genotype, type of cocoa trees and experimental combinations were sources of variation. Variances were estimated using SAS (SAS Institute, 1988) software, with two factors (cocoa genotypes and experimental combinations). A general linear model type III was performed because of the unbalanced data, and a mean values of individual genotypes *post hoc* test was performed using least square mean (LSM) and Newman–Keuls tests. This analysis was performed on the data without transformation, because the conditions for the ANOVA were satisfied.

3 RESULTS AND DISCUSSION

3.1 Microtest observations

3.1.1 Step 1

Genotype ranking results by score when tested in combinations and by LSM number of feeding points are given respectively in Tables 2 and 3.

The genotypes BE10 and SNK413 showed the highest score in the combinations where they were associated. On the other hand, SIC5 and IMC60 showed the lowest score in combinations (Table 2). Analysis of variance on the LSM number of feeding points revealed a highly significant effect of the genotype ($F = 3.15$, $P < 0.0006$). The Newman–Keuls test showed three significantly different groups (Table 3). BE10 had the highest number of feeding points, and consequently can be considered as the most attractive genotype. On the other hand, IMC60 showed a lower number of feeding points than SNK413 and BE10, and in this way a lower attractiveness towards mirids.

Ranking by score when tested in combinations and ranking by LSM number of feeding points are

Table 2. Ranking of the attractiveness of international cocoa genotypes based on the average number of mirid feeding points in triangular microtest combinations. Each genotype was tested 3 times. For the combinations: 1 = first position (highest number of feeding points); 2 = second position; 3 = third position (lowest number of feeding points)

Cocoa genotype	Mean number of feeding points	Combinations and the order of attractiveness 1	2	3	score	Total Rank
BE10	4.96	1	1	1	3	1st
SNK413	4.45	1	1	1	3	
SNK614	2.98	2	1	1	4	3rd
T60/887	3.60	2	2	1	5	4th
UPA134	3.55	2	2	1	5	
SNK619	3.20	3	1	2	6	6th
PA107	3.09	3	2	2	7	8th
Playa Alta2	2.69	2	2	3	7	
Catongo	2.56	3	3	2	8	9th
SIC5	2.87	3	3	3	9	10th
IMC60	2.44	3	3	3	9	

Table 3. Comparison of means for eleven international cocoa genotypes tested for mirid feeding points from Newman–Keuls grouping. Mean values for individual genotypes are given in Table 2

Cocoa genotype	Number of observations	Newman–Keuls grouping		
BE10	45	A		
SNK413	42	A	B	
T60/887	45	A	B	C
UPA134	42	A	B	C
SNK619	45	B	C	
PA107	42	B	C	
SNK614	45	B	C	
SIC5	45	B	C	
PLAYA ALTA2	45	B	C	
CATONGO	45	B	C	
IMC60	45	C		

slightly different. For instance, SNK614 was ranked among the worst genotypes, with the third score in combinations, and yet it showed a medium position in the LSM ranking. The reason is that it was combined with couples of genotypes less attractive than itself in two combinations (combination 7 and 8 in Table 1). SIC 5 showed an opposite ranking.

To improve the confidence in ranking, it is suggested that both the average number of mirid feeding points and the score in combinations be taken into account.

Analysis of variance revealed no trends in the attractiveness of the type of cocoa tree (Upper Amazonian, Lower Amazonian, Trinitario), as shown in Tables 2 and 3.

3.1.2 Step 2

An estimate of the average number of feeding points of tested genotypes gave a mean of 3.30. Five genotypes therefore showed a higher score than the mean, and six would be ranked below this value. At this step, to estimate distances between each genotype value and average value, use was made of the result of the Newman–Keuls test of homogeneity which discriminated three groups (A, B and C). The intermediate genotype placed in each grouping was considered to be the one with medium attractiveness. The highest score of that group was the score of T60/887 (3.60) at a distance $\Delta = 0.30$ from μ. Therefore, the average became $\mu = 3.30 \pm 0.30$, with four genotypes grouped around it (Table 3).

In 1998, Nguyen-Ban[21] conducted triangular microtests with 21 genotypes coming from the Nkoemvone research station in Cameroon. He proposed a ranking in three categories according to the difference in attractiveness of genotypes towards the genotype SCA6, which was chosen as a control. In this way the author found six more attractive genotypes and six less attractive genotypes than SCA 6, and nine not significantly different genotypes. Among the 21 genotypes assessed previously, only two were tested in the present study: UPA 134, which is categorised as more attractive than SCA 6, and SNK 413, which did not show any significant difference from SCA6.

Comparison is easier with results obtained by Babin *et al.*[16] in 2004, who compared cocoa genotypes for their antixenosis towards mirids. Nymphs were confined on a flush in a nylon mesh sleeve cage and allowed to feed on the plant over a 24 h period. Nymphs were therefore not in a position to choose their diet as in the present study. Even so, the ranking of the average number of feeding points showed similarities: BE10 was among the most bitten genotypes, while IMC60, CATONGO and SIC5 were the least attacked. The authors proposed an assessment of the tolerance by examining the physiological reaction of the flush and its ability to recover from damage. BE10 was among the less tolerant genotypes, while IMC60 was the most tolerant to mirid attack.

4 CONCLUSIONS

In this paper, the authors proposed to better Nguyen-Ban's method by using a circular microtest that avoids the use of a control. This relatively user-friendly method might be seen as a tool for cocoa genotype selection for mirid attacks. The results confirmed that attractiveness is an important component of cocoa resistance to mirids, and this character could be considered in an early stage of the breeding process. Comparison with a tolerance study[16] revealed that attractiveness and tolerance might be linked components according to the similar ranking results.

This observation has to be confirmed by a study of insect–plant relationship mechanisms. The results presented in this paper made it possible to identify some promising genotypes for the attractiveness component of cocoa resistance to mirids: IMC 60, CATONGO and SIC5. The genotype BE10 is unsuitable for regions where cocoa mirids are numerous. These results have to be confirmed by plot experimentations; in particular, the question as to whether attractiveness is a principal or a slight component of resistance/tolerance at field level must be addressed.

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