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Relationship of phenotypic structures and allelochemical compounds of okra (*Abelmoschus* spp.) to resistance against *Aphis gossypii* Glover

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The cotton aphid (*Aphis gossypii*) is one of the major pests of okra. Eleven okra varieties collected from farmers' fields in Cameroon and four aphid-resistant accessions from The World Vegetable Center (AVRDC, Asian Vegetable Research and Development Center) were evaluated from October 2012 to March 2013, and another seven aphid-resistant accessions from AVRDC were evaluated from March to July 2013 at AVRDC's Nkolbisson station, Yaoundé, Cameroon. Accessions selected from these trials were screened in an advanced confirmatory screening in autumn (October−December) 2013 in Shanhua, Taiwan. Results showed that none of the farmers' landraces were resistant to aphids. Two accessions (VI041210 and VI033824) with the lowest aphid populations had the highest trichome density. Analysis of various phytochemicals in selected okra accessions revealed that higher nitrogen and potassium levels made the okra accessions susceptible to aphids during the reproductive stage of the plants. Similarly, the total phenol content was lower in moderately resistant accessions during their reproductive stage. Aphid feeding induced an increase in phenols, and to a lesser extent, tannins. Accessions VI041210 and VI033824 could be incorporated into an integrated pest management strategy to enhance productivity of okra.

Keywords: resistance; okra; aphids; biophysical and biochemical bases; feeding-induced responses

1. Introduction

Okra belongs to the *Abelmoschus* genus in the family of Malvaceae. It was formerly included in the genus *Hibiscus*. Cultivated species are West African okra [*Abelmoschus caillei* (A. Chev.) Stevels], common okra [*Abelmoschus esculentus* (L.) Moench.], aibika [*Abelmoschus manihot* (L.) Medik.] and a fourth domesticated species *A. moschatus*. *A. manihot* usually does not flower and it is extensively cultivated for its leaves in Papua New Guinea, Solomon Islands and other South Pacific Islands (Kumar et al. 2010). *A. moschatus* is cultivated for culinary as well as medicinal purposes in India, South Togo and Benin (Hamon & Sloten 1995). Okra (*A. esculentus* and *A. caillei*) is an important vegetable in South and Southeast Asia and sub-Saharan Africa. Okra is produced in an area of over 1 million hectare worldwide, with Asia and Africa having about 0.5 Mha each (FAO 2012). India is the world's leading okra producer (6 million t annually), followed by Nigeria (1.1 million t). Cameroon produces about 64,000 t annually in an area of about 26,500 ha. Asia and Africa have almost similar acreage under okra production, but the average productivity in Africa is very low (3.55 t/ha) compared to Asia (11.52 t/ha). Although several factors contribute to the low productivity of okra in Africa, biotic constraints are a major limiting factor.

The cotton or melon aphid, *Aphis gossypii* Glover (Homoptera: Aphididae) is one of the major constraints to okra production in Cameroon. *A. gossypii* is found in tropical and temperate regions of the world, except in extreme northern areas. It has a wide range of almost 700 host plants worldwide (Capinera 2000). Besides causing direct damage, *A. gossypii* is known to transmit more than 50 plant viruses (Blackman & Eastop 2007). The cotton aphid usually infests the underside of the okra leaves. In severe infestations, the pest is also found on the stems, fruits and upper leaf surface. Aphids cause damage either directly by feeding, which results in curling and deformation of young leaves and stems, or indirectly by contaminating the leaves and fruits with honeydew, which favours the growth of black sooty mould that inhibits photosynthesis, thus causing substantial yield loss (Capinera 2000). Honeydew attracts ants that fend off natural enemies of aphids (Yokomi & Tang 1995). Chemical treatment remains the main method of control, although resistance to insecticides has been documented in many *A. gossypii* populations (Owusu & Yeboah 2007). Hence, alternative pest management strategies are needed.

Plant resistance is an important component in integrated pest management (IPM) strategies against aphids. Plant morphological characters play a vital role in imparting resistance to various insect pests. Although we do not find many references on the importance of plant characteristics in okra offering resistance to *A. gossypii,* evidences are available in cotton, another Malvaceous species.

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Trichomes in cotton and okra have either adverse (Zarpas et al. 2006; Abang et al. 2014) or positive effects (Nibouche et al. 2008) on resistance to *A. gossypii*. Morphological or structural characteristics such as silica content, leaf toughness, deceptive plant structures and leaf size in cotton also enhance plant resistance (Deguine & Hau 2001). These morphological characters influence aphids' settling and feeding behaviour. For instance, after 72 h of infestation, most of the *A. gossypii* left the leaves of resistant melon plants, since they found them unsuitable for feeding and colonization (Soria et al. 2000). However, we did not find any such preference or avoidance behaviour on resistant and susceptible okra accessions in our earlier study (Abang et al. 2014). On an aphid transmitted virus resistant melon plant, *A. gossypii* seldom reached the phloem, or stopped feeding when phloem was reached (Klingler et al. 1998), and then starved.

Plants produce biochemicals that are involved in plant defence. For instance, terpenoids are involved in defence as toxins and feeding deterrents; they are also common in plants and are used as active ingredients of pesticides (Turlings et al. 1995). Phenols, most commonly tannins, act as toxins and repellents, and also bind to insect salivary proteins. Ma et al. (2005) reported significant feeding inhibitions to *A. gossypii* on artificial diet containing tannic acid, since it reduced the total probing time. Secondary metabolites containing nitrogen and sulphur, such as alkaloids and non-protein amino acids, act as protein inhibitors, deactivators, toxic substances and irritants. Plants also produce volatile organic compounds, especially during induced resistance, to repel herbivores and attract natural enemies (Dicke &and Van Loon 2000; De Moraes et al. 2001; Kessler & Baldwin 2001). Hegde et al. (2012) confirmed that antixenosis in cotton was activated by the natural plant defence elicitor cis-jasmone in the presence of *A. gossypii*. The levels of free amino acids and sugars in cotton may partially determine the likelihood of *A. gossypii* infestation (Deguine & Hau 2001). Plant nitrogen content is an indicator of food quality and host selection by *A. gossypii*, since amino acids are important for the development of *A. gossypii* (Slosser et al. 1989).

Since host plant resistance to *A. gossypii* based on biophysical and bio-chemical characteristics in Malvaceous species such as cotton has been confirmed, we aimed to screen the okra germplasm for their resistance to *A. gossypii*. Hence, the objectives of this study were (1) to identify the *Abelmoschus* spp. genotypes that are resistant to *A. gossypii* in Cameroon and (2) to characterize the biophysical and biochemical bases of aphid resistance that lead to antixenosis and antibiosis.

2.   Materials and methods

2.1.   *Field screening of okra germplasm against aphids*

Our earlier study confirmed the genetic similarity of *A. gossypii* populations in Taiwan and Cameroon based on the mitochondrial cytochrome c oxidase I (*coxI*) phylogenetic analysis (Abang et al. 2014). Hence, shuttle screening of the okra germplasm was carried out in Taiwan as well as Cameroon to expedite the identification of aphid resistant okra accessions. Eleven okra varieties collected from farmers' fields in Cameroon, and four accessions rated as aphid resistant from a non-replicated preliminary screening trial in Taiwan that involved a total of 107 accessions were evaluated in a replicated trial from October 2012 to March 2013. A known susceptible accession (VI057245) was also included. Subsequently, seven accessions rated as aphid resistant from another non-replicated preliminary screening trial involving 64 accessions in Taiwan were evaluated in the second replicated trial from March to July 2013. These two trials were conducted in Nkolbisson, Yaoundé, Cameroon (03°51.79′N and 11°27.71′E). Accessions selected from these two trials including the susceptible accession were screened in an advanced confirmatory trial in October 2013−January 2014 in Shanhua, Taiwan. The seeds were sown on 5th October and a plant to plant spacing of 1 m, and a row to row spacing of 1.5 m were maintained. These trials were conducted using a randomized block design with three replications. The trials were maintained following customary cultural practices, and without pesticide application to control aphids or other sucking insects to increase the natural aphid infestation. Aphid populations were directly scored at weekly intervals starting from four weeks after transplanting in the field. The aphid population was scored on three leaves (one each from the bottom, middle and top) of 10 randomly selected plants in each replication, using the following rating scale: $0 =$ no aphids present; $1 = 1−10$ aphids per leaf; $2 = 11−100$ aphids per leaf; $3 = 101−1000$ aphids per leaf; and $4 = >1000$ aphids per leaf. When the aphids were less than 100, they were directly counted on selected leaves. However, the aphids were collected from selected leaves and counted, when the colonies had high number of aphids. The scored data from each accession was expressed as the area under the infestation pressure curve (AUIPC) and calculated using the following formula modified from Shaner and Finney (1977):

$$\sum_{i=1}^{n-1} \frac{(Y_i + Y_{i+1})}{2} (t_{i+1} - t_i)$$

where $n =$ number of assessment times and $Y =$ number of insects at time t.

2.2.   *Biophysical and biochemical bases of resistance*

2.2.1.   *Biophysical bases of resistance*

The biophysical bases of resistance, viz., leaf trichome density (Bourland et al. 2003) and toughness were examined in selected resistant and susceptible accessions (VI033824, VI039614, VI041210, VI060688, VI060794, VI060818 and VI057245) (Abang et al. 2014). Three leaves, one each from the bottom, middle and top of the plant, were collected at the beginning of flowering. For leaf trichome density, 1 cm^2 leaf pieces were collected

from either side of the leaf mid-vein. The leaf pieces were mounted on a stereomicroscope and the number of trichomes was counted. For measuring the leaf toughness, 1 cm^2 leaf pieces were collected from either side of the mid-vein and mounted on a gram gauge. A 0.52-mm diameter blunt probe was used to measure the force required to puncture leaf tissues. The gram gauge was designed by modifying a scale balance using a method described by Wheeler and Center (1997). Measurements of leaf toughness and trichome densities were recorded in five replicates with two plants per replicate for each accession.

2.2.2.   *Biochemical bases of resistance*

Tannins, total sugar, reducing sugars, phenolic compounds, total nitrogen and potassium were estimated in okra leaves of selected resistant and susceptible accessions (Abang et al. 2014). The plants were grown in a controlled environment with aphid infestation (for induced response) and without aphid infestation (for constitutive resistance), following the completely randomized design. Two separate plants were used at 6 and 10 weeks after sowing (vegetative and reproductive stage) (Shannag et al. 2007). To study induced response, 10 aphids at 6 weeks and 25 aphids at 10 weeks were released on each plant to feed and reproduce for 5−6 days following the protocol of Messina and Bloxham (2004). All aphids on infested plants were counted to estimate aphid density at the end of the infestation. Six leaves from infested and uninfested plants (two each from the top, middle and bottom of the plant) were collected and dried at −56 °C for seven days in a freeze dryer. The dry samples were ground in an electric blender. The samples were replicated five times for each accession, with two plants per replication.

Analysis of tannins in leaves of aphid-resistant and susceptible okra accessions was conducted using the Catechin standard and acidified vanillin method (Broadhurst & Jones 1978). One gram of powder sample per replication was used for analysis. Total sugar content of leaves in aphid-resistant and susceptible okra accessions was spectrophotometrically determined using Anthrone reagent (Dreywood 1946). Anthrone reagent reacts specifically with carbohydrates in a concentrated sulphuric acid solution to produce a blue-green colour at 630 nm. The results were expressed as sucrose equivalents. Reducing sugar content was determined using the Nelson−Somogyi method (Somogyi 1952). Reducing sugars, when heated with alkaline copper tartrate, reduce the copper from the cupric to cuprous state and thus cuprous oxide is formed. When cuprous oxide is treated with arsenomolybdic acid, the reduction of molybdic acid to molybdenum blue takes place. The resulting blue colour is compared with a set of standards in a colorimeter at 620 nm. The colorimetric method of Folin–Denis as described by Swain and Hillis (1959) was used to determine the phenolic compounds in the leaves. Total leaf nitrogen was determined following Kjeldahl's method as described by Bremner and Mulvaney (1982). The potassium content in the digested solution

of plant tissues was diluted and analysed by flame-photometer as described by Rayment and Lyons (2011).

2.3.   *Antixenosis*

Antixenosis or non-preference was investigated by studying the settling behaviour of aphids. Plants were infested with 10 aphids, after two hours of starvation, on the upper surface of the second fully expanded leaf from the apex. After 72 h, adult aphids were counted to evaluate their permanence on the infested leaf, and leaves with four or less aphids were considered non-preferred by aphids (Fereres, 1994).

2.4.   *Statistical analysis*

The AUIPC values for aphid population per leaf were subjected to a statistical analysis based on mean (m) and standard deviation (sd) and categorized as follows: the accessions that had mean damage score (n) less than $m − 2$sd were considered highly resistant; between $m − 1$sd to $m − 2$sd as resistant; between m to $m − 1$sd as moderately resistant; between m to $m + 1$sd as moderately susceptible; between $m + 1$sd to $m + 2$sd as susceptible; and more than $m + 2$sd as highly susceptible.

Data obtained from experiments on the biochemical and biophysical traits of selected okra accessions were subjected to analysis of variance (ANOVA) with the Proc GLM procedure of SAS, version 9.1 (SAS Institute, Cary, NC, USA). The choice test on the settling behaviour of aphids was analysed using the Kruskal–Wallis test with one-way ANOVA and non-parametric test. If significant differences were found between treatments, Tukey's honestly significant difference (HSD) test was used as a *post hoc* procedure for comparison.

3.   Results
3.1.   *Field screening of okra germplasm against aphids*

Out of the 16 varieties/accessions screened in the first trial (October 2012 to March 2013), only 1 accession (VI033824) was rated as resistant, whereas 10 accessions/varieties were moderately resistant (Table 1). None of the farmers' varieties were resistant. In the second trial (March−July 2013), out of seven accessions, five were moderately resistant and none were resistant. Results of the advanced confirmatory screening trial during November 2013 to January 2014 showed that four accessions (VI041210, VI057245, VI033824 and VI060818) including the susceptible accession were moderately resistant (Table 1).

3.2.   *Biophysical and biochemical bases of resistant accessions*

3.2.1.   *Biophysical bases*

There were significant differences in leaf trichome density in leaves from top ($P < 0.0001$), middle ($P = 0.005$) and

Table 1.   Area under the infestation pressure curve (AUIPC) of okra accessions assessed for aphid numbers from October 2012 to January 2014.

October 2012 to March 2013		March−July 2013		October 2013 to January 2014	
Accession	AUIPC (*n*)	Accession	AUIPC (*n*)	Accession	AUIPC(*n*)
VI033824	474.3 R	VI060818	594.9 MR	VI041210	42.9 MR
VI033796	516.0 MR	VI060794	625.7 MR	VI057245	43.5 MR
Bityili giant	544.4 MR	VI060688	640.5 MR	VI033824	49.8 MR
Njombe caffeier	551.1 MR	VI041210	656.2 MR	VI060818	51.8 MR
Maroua	582.3 MR	VI039614	808.4 MR	VI060688	58.5 MS
VI033778	671.0 MR	VI060866	1015.3 MS	VI039614	67.1 MS
VI057245[1]	684.1 MR	VI060817	1415.7 S	VI060794	82.2 S
Small soppo	696.5 MR				
Evodoula six months	705.4 MR				
Munya buea	755.9 MR				
VI046537	763.4 MR				
Baba I	785.5 MS				
Njombe red	929.1 MS				
Ebebda green	1015.5 MS				
Njombe green	1219.2 S				
Babungo	1519.8 HS				
Mean (*m*)	775.8		822.4		56.6
Sd	278.4		300.2		14.3
m − 2sd	219.1		222.1		28.0
m − sd	497.5		522.2		42.3
m + sd	1054.2		1122.5		70.9
m + 2sd	1332.5		1422.7		85.2

[1]Susceptible check.
Note: R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible and HS = highly susceptible.

bottom ($P = 0.0004$) strata among the accessions. In general, the trichome density was significantly higher in the top three moderately resistant accessions (VI041210, VI057245 and VI033824) than in the other accessions (Table 2). The trichome density was significantly lower in the susceptible accession (VI060794). For leaf toughness, there were no significant differences among the accessions for the force needed to puncture the leaves from top ($P = 0.41$), middle ($P = 0.11$) and bottom ($P = 0.14$) strata (Table 2).

3.2.2.   *Biochemical bases*

Biochemical studies of selected okra accessions at six weeks after sowing showed that there were significant differences between accessions in total phenols ($P = 0.022$) and total tannins ($P = 0.017$) of plants without aphids, but not in plants with aphids. Total phenols were higher in plants infested with aphids than in plants without aphids; the opposite was observed for total tannins (Table 3). Although one of the top ranking moderately resistant

Table 2.   Leaf hairiness and hardness in selected okra accessions.

	Leaf trichome density (no/cm^2)			Leaf toughness (g)*		
Accession	Bottom	Middle	Top	Bottom	Middle	Top
VI060794[1]	0.67 b	1.42 c	0.92 d	7.28	10.88	12.18
VI039614	1.33 b	3.75 abc	11.25 bc	8.35	09.27	12.37
VI041210	2.67 ab	5.75 ab	20.50 a	7.28	11.85	13.53
VI057245	2.67 ab	6.67 a	11.75 bc	6.58	10.95	10.95
VI060688	1.08 b	1.92 bc	6.83 cd	10.6	14.05	13.78
VI060818	1.58 b	2.33 bc	5.58 cd	7.52	10.55	10.33
VI033824	4.92 a	4.83 abc	15.25 ab	9.80	11.04	13.35
F-value	8.83	5.16	14.15	1.94	2.20	1.09
Treatment and error df	6, 28	6, 28	6, 28	6, 28	6, 28	6, 28
P-value	0.0004	0.005	<.0001	0.14	0.11	0.41

[1] Susceptible check.
Note: Values represent the force (g) needed to puncture the leaf.
Means with the same letter in a column are not significantly different at $P < 0.05$ by Tukey's HSD.

Table 3. Biochemical content of okra accessions with and without aphids at vegetative growth (six weeks after sowing).

Biochemical	Aphid	VI060818	VI057245	VI060794[1]	VI060688	VI041210	VI039614	VI033824	$F_{(6, 28\text{ df})}$-value	P-value
Phenols (mg/100 g)	No	1142 ab	1436 a	1229 ab	1173 ab	1073 b	1090 b	1072 b	3.64	0.022
	Yes	1537	1432	1508	1461	1355	1442	1645	0.50	0.795
Tannins (mg/g)	No	0.56 ab	0.52 b	0.62 a	0.62 a	0.60 ab	0.55 ab	0.58 ab	3.92	0.017
	Yes	0.53	0.64	0.56	0.54	0.49	0.50	0.55	1.18	0.372
Total sugars (mg/g)	No	1.29	1.30	1.29	1.30	1.31	1.30	1.31	2.32	0.091
	Yes	1.30	1.31	1.30	1.30	1.30	1.30	1.30	0.78	0.597
Reducing sugars (%)	No	23.20	27.90	16.60	19.50	26.50	22.70	21.00	1.97	0.138
	Yes	18.90 c	42.10 ab	22.70 c	24.90 bc	35.50 abc	46.80 a	24.20 bc	3.40	0.028
Total nitrogen (%)	No	3.90	3.50	4.10	4.00	3.80	3.90	4.30	2.50	0.074
	Yes	4.10 a	3.40 b	4.00 a	3.80 ab	3.40 b	3.80 ab	4.10 a	8.16	0.0006
Potassium (%)	No	2.16 b	2.73 a	2.79 a	2.00 b	2.59 a	1.95 b	2.17 b	22.78	<0.0001
	Yes	2.12 c	2.46 a	2.33 ab	1.69 e	2.21 bc	1.81 de	1.86 d	32.07	<0.0001
Aphid load after five days (10 adult aphids)		118.60	197.40	160.20	196.30	381.10	223.80	166.30		

[1] Susceptible check.
Note: Means with the same letter in a row are not significantly different at $P < 0.05$ by Tukey's HSD.

accessions (VI057245) had higher total phenol, it was not significantly higher than the susceptible accession VI060794. The other two top ranking moderately resistant accessions (VI041210 and VI033824) had less total phenol, but were comparable with the susceptible accession (VI060794). Hence, the total phenol content among the accessions did not reveal any clear relationship with aphid resistance. When the plants were not infested with aphids, one of the top ranking moderately resistant accessions (VI057245) had significantly lower tannins than the susceptible accession VI060794. However, the tannin content among the other accessions did not reveal any clear relationship with aphid resistance. Total sugars showed no significant differences between accessions infested with or without aphids. However, when infested with aphids, the reducing sugar content was higher in one of the moderately resistant accessions (VI057245). There were significant differences in total nitrogen among the selected okra accessions, infested with aphids ($P = 0.0006$), and two moderately resistant accessions (VI057245 and VI041210) had significantly lower nitrogen content than the susceptible accession. However, potassium content did not show any relationship with aphid resistance in selected okra accessions.

At 10 weeks after sowing, there were significant differences in total phenols among the okra accessions, only for plants infested with aphids ($P = 0.036$) (Table 4). Unlike the younger plants, okra accessions at this stage clearly showed the difference in total phenols among the resistant and susceptible accessions. For instance, total phenols were significantly lower in three moderately resistant accessions (VI041210, VI057245 and VI033824) than in the susceptible accession (VI060794). For tannins, significant differences were found between the accessions in both plants infested with ($P = 0.009$) or without ($P = 0.007$) aphids. However, a clear difference in tannins among the susceptible and resistant accessions did not exist, except in VI041210 (plants without aphids), which had significantly lower levels of tannins than the susceptible accession. Total sugars also showed significant differences between accessions with ($P = 0.025$) and without ($P = 0.0003$) aphids, but only VI033824 (without aphid infestation) had significantly higher total sugars than the susceptible accession. Total nitrogen showed significant difference between accessions without aphids ($P < 0.0001$) only. The susceptible accession (VI060794) had significantly higher total nitrogen than other accessions. Similarly, potassium content in the susceptible accession was also higher than the moderately resistant accessions, in both with or without aphid infestation.

3.3. *Antixenosis*

The results indicated that moderately resistant accessions did not induce antixenosis There were no significant differences between the accessions in the number of aphids per leaf after 72 h of release ($P = 0.6861$). Thus, aphid number did not differ on susceptible and resistant okra accessions (Table 5).

Table 4. Biochemical content of okra accessions with and without aphids at reproductive growth (10 weeks after sowing).

Biochemical	Aphid	VI060794[1]	VI060818	VI060688	VI039614	VI033824	VI057245	VI041210	$F_{(6,\ 28\ df)}$-value	P-value
Phenols (mg/100 g)	No	1624	1866	1585	1359	1264	1633	1564	1.70	0.194
	Yes	2171 a	1481 a	2117 a	1765 ab	1602 b	1564 b	1537 b	3.14	0.036
Tannins (mg/g)	No	0.44 a	0.43 a	0.40 ab	0.40 ab	0.40 ab	0.36 ab	0.33 b	4.81	0.007
	Yes	0.46 ab	0.47 ab	0.43 b	0.47 ab	0.49 a	0.42 b	0.46 ab	4.53	0.009
Total sugars (mg/g)	No	1.29 b	1.30 b	1.29 b	1.30 ab	1.32 a	1.30 b	1.29 b	9.22	0.0003
	Yes	1.29 b	1.29 ab	1.29 ab	1.30 ab	1.31 a	1.30 ab	1.29 b	3.50	0.025
Reducing sugars (%)	No	18.00 c	37.60 b	37.00 b	38.40 ab	55.90 a	38.80 ab	26.20 bc	4.50	0.0096
	Yes	15.00 d	24.20 cd	27.60 cd	42.30 b	65.00 a	38.10 bc	29.70 bc	13.20	<0.0001
Total nitrogen (%)	No	3.31 a	2.90 b	2.90 b	2.44 c	2.77 b	2.46 c	2.54 c	19.49	<0.0001
	Yes	2.90	3.08	2.96	2.75	2.90	2.79	2.92	0.83	0.56
Potassium (%)	No	2.59 a	1.76 cd	1.68 cd	1.28 e	1.54 d	2.06 b	1.92 bc	33.3	<0.0001
	Yes	2.37 a	2.00 c	1.64 d	1.42 e	1.56 de	2.19 b	2.20 ab	41.2	<0.0001
Aphid load five days after (25 adult aphids)		677.30	321.10	396.00	435.70	173.30	227.30	384.70		

[1] Susceptible check
Note: Means with the same letter in a row are not significantly different at $P < 0.05$ by Tukey's HSD.

Table 5. Settling behaviour of aphids on selected okra accessions.

Accession	Non-preference (%)	Mean number of aphids per leaf after 72 h
VI060794[1]	22.22	8.10
VI039614	11.11	7.80
VI041210	11.11	10.30
VI057245	11.11	8.70
VI060688	11.11	8.30
VI060818	11.11	7.60
VI033824	0.00	8.10
$X^2_{6\ df.}$		3.931
Pr > Chi-square		0.6861

[1] Susceptible check.

4. Discussion

This study included several popular okra varieties collected from farmers' fields in Cameroon. However, none of these varieties were resistant to *A. gossypii*. This indicates that the pest status of *A. gossypii* on okra is high in Cameroon because of the lack of resistant varieties. Out of the seven accessions screened in the confirmation trials, four accessions (VI041210, VI057245, VI060818 and VI033824) were found to be moderately resistant. Although VI057245 was included as the known susceptible accession, it was consistently moderately resistant in both the trials in Cameroon and the final trial in Taiwan. This suggests that resistance to aphids may vary in space and time. Besides, the resistance may also vary depending on the host race, because *A. gossypii* is structured into geographically widespread host races specialized on Cucurbitaceae, cotton, eggplant or pepper (Carletto et al. 2010). We used the race from okra, which may be similar to cotton because they belong to Malvaceae. However, the selected okra accessions could display different reactions to non-cotton or okra races of *A. gossypii*, if they are present in a location where the selected resistant accessions are screened. Hence, repeated screening trials should be conducted before discarding the germplasm materials. VI041210 was an old cultivar, collected at Cabaroan (17°39′N, 120°22′E), Santa Catalina, Ilocos Sur province in the Philippines. VI057245 was a landrace collected at Cham Ko Louk (13°26′56.1 "N, 103°00′13.5"), in Banteay Meanchey province of Cambodia. VI060818 was a local cultivar (ORS 354) from Mali, whereas VI033824 was an old cultivar collected at Koronadal, Barrio (06°30′ N, 124°50′ E) in South Cotabato province of the Philippines. All these accessions were the Asian okra species of *A. esculentus*. Our earlier study revealed that *A. esculentus* accessions originating from the Philippines possess appreciable levels of resistance to *A. gossypii* (Abang et al. 2014). However, it is important to evaluate their adaptation to Cameroon conditions in future studies to confirm their yield potential before recommending them for farmers' use.

Although West African okra (*A. caillei*) accounts for only 5% of the total world okra production (Siemonsma

& Kouame 2004), it is a very important crop in the major okra producing countries in Africa. However, it has to be noted that the aphid resistant accessions identified in the current study were only the Asian okra species *A. esculentus*. Kumar et al. (2010) suggested that earliness of *A. esculentus* as compared to *A. caillei* was preferred in the Sudano-Sahel. Hence, these aphid resistant accessions could be directly cultivated in these regions after local validation. However, the adaptability of these *A. esculentus* accessions should be confirmed before promotion, because *A. caillei* has gradually replaced *A. esculentus* in the tropical-humid region because of its better adaptation under humid zone (Siemonsma 1982). In addition, aphid resistant *A. esculentus* accessions can also be used in resistance breeding programs, since inter-specific cross between *A. caillei* and *A. esculentus* is successful with the possibility of gene transfer, although the partial hybrid breakdown barrier must be overcome (Fatokun 1987). However, the use of okra accessions identified in this study as parental lines in breeding programs may not be advisable, since the aphid resistance is only moderate.

The biophysical traits of the plants play an important role in the colonization, feeding and development of *A. gossypii* on okra. Trichomes in cotton and okra have either adverse (Zarpas et al. 2006; Abang et al. 2014) or positive effects (Nibouche et al. 2008) on resistance to *A. gossypii*. In the present study, the biophysical bases (such as leaf trichomes) were significantly different between resistant and susceptible accessions. Three top-ranking moderately resistant accessions that had lower AUIPC values had higher trichome densities. This suggests that leaf trichomes are one of the factors that influence the colonization of *A. gossypii* on okra plants. Similar observations were made in our earlier study (Abang et al. 2014) as well as by Santos et al. (2003) and Soglia et al. (2002, 2003). Although both glandular and non-glandular types of trichomes have been described in the genera *Sida* and *Hibiscus* of Malvaceae (Shaheen, Khan, et al. 2009; Shaheen, Ajab, et al. 2009), only three non-glandular trichome types (unicellular filiform hairs, unicellular conical hairs and stellate hairs) have been described in *Abelmoschus* (Osawaru et al. 2011). Since we did not study the types of trichomes in these selected moderately resistant accessions, detailed studies may be carried out in the future, if one wants to exploit these accessions. Some studies showed that *A. gossypii* populations prefer young and succulent leaves, which are generally located on the apical parts of susceptible varieties (Santos et al. 2003; Chau et al. 2005). Non-occurrence of aphids on the apical leaves of resistant varieties is an indication that these leaves have more trichomes, which can make locomotion, feeding and reproduction of *A. gossypii* difficult (Soglia et al. 2002, 2003; Santos et al. 2003). In the current study also, significantly higher number of trichomes were found in the leaves from the top stratum among the three studied strata. Hence, trichome density in the top strata of the plants may be considered while selecting the accessions for their resistance to *A. gossypii* in the future. In addition to trichomes, leaf hardness is important in enhancing plant

resistance (Deguine & Hau 2001). However, our current study did not find any evidence supporting the influence of leaf hardness against aphid infestation on resistant or susceptible accessions.

Plant chemistry also affects pest infestations. Biochemical studies of selected okra accessions at 6 and 10 weeks after sowing showed that total phenols were higher in plants infested with aphids than in plants without aphids in most accessions. It has been reported that some of the traits and processes that defend plants against pests change following pest attacks (Khattab 2007; Wilson et al. 2011). The susceptible accession had higher total phenols than the moderately resistant accessions during the reproductive stage of okra. Zucker (1982) found an inverse correlation for the effect of total phenols in *Populus anqustifolia* to the galling aphid, *Pemphigus betae*. However, our results are contrary − total phenol content is positively correlated with the aphid infestation. This was not the case in our earlier study, in which the leaf total phenols did not play a significant role in imparting resistance in okra to aphids (Abang et al. 2014). In addition, when there was an aphid infestation, the plant defence compounds, especially phenolic compounds, were increased. A similar rapid increase in total phenols following insect damage was recorded by Tuomi et al. (1988). As documented in our previous study (Abang et al. 2014), our current study also confirmed that tannins did not have any significant role in imparting resistance to *A. gossypii* in okra accessions, although Ma et al. (2005) found that tannins affected *A. gossypii* feeding. Total sugars and reducing sugars did not have a significant role in offering resistance in plants infested with or without aphids at all growth stages. However, total nitrogen at all growth stages and potassium at the reproductive stage of okra were higher in the susceptible accession. Although Leite et al. (2007) confirmed that leaf nitrogen and organic compounds in okra did not have any effects on *A. gossypii* populations, and Lu et al. (2009) found that total nitrogen and amino acids in cotton were not associated with resistance to *A. gossypii*, the current study revealed that the total nitrogen increased the susceptibility of okra accessions to aphids.

The number of aphids did not differ between the okra accessions in the antixenosis study. However, leaf trichome density was lower, and nitrogen and potassium content were higher in leaves of susceptible VI060794 than in the moderately resistant accessions. These parameters did not affect aphid settling behaviour, but may be responsible for subsequent population build-up on the susceptible accession. It should also be noted that VI060794 was not a highly susceptible accession, and it showed a moderate resistance in a trial in Cameroon during March–July 2013. Since this was a newly initiated aphid resistance screening programme involving the okra germplasm from The World Vegetable Center (AVRDC, Asian Vegetable Research and Development Center) − and we did not have a well-known aphid susceptible okra line, we chose one of the susceptible accessions from the available list. Hence, some of the results from bio-chemical studies

differ from earlier studies on other crops or in-conclusive, and hence need further confirmation. Future studies on antibiosis will certainly help to explain the difference in aphid infestation levels.

5. Conclusions

Four okra accessions (VI041210, VI057245, VI060818 and VI033824) were identified as moderately resistant to aphids. Leaf trichome density especially on leaves from top stratum plays a major role in okra resistance to aphids. Two accessions (VI041210 and VI033824) with the lowest aphid population, highest trichome density and lower leaf nitrogen content are potential sources of resistance to *A. gossypii* in okra. The primary metabolites and plant nutrients did not change with aphid infestation, except secondary metabolites such as phenols and tannins. It seems that aphid feeding induced a response not related to okra primary metabolites and minerals, although it needs further confirmation. The effects of plant parameters did not affect aphid settling behaviour. Accessions VI041210 and VI033824 could be incorporated into IPM strategies to enhance productivity, incomes and livelihoods of okra farmers.

Disclosure statement

No potential conflict of interest was reported by the authors.

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