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Identification of okra (*Abelmoschus* spp.) accessions resistant to aphid (*Aphis gossypii* Glover) in Cameroon

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Okra germplasm collected from different locations around the world were screened at AVRDC (The World Vegetable Center) in 2011 and 2012 to identify germplasm resistant to the melon aphid (*Aphis gossypii*) for use in sub-Saharan Africa. A total of 260 okra accessions and varieties were screened (150 at AVRDC Taiwan and 110 at AVRDC Cameroon), which included four varieties commercially available in Cameroon. The experiments were conducted under natural infestation in Shanhua, Taiwan, and at Yaoundé, Cameroon. Since the preliminary screening trials were conducted in Taiwan and Cameroon, the aphid populations in these two countries were compared. A total of 60 insects was used for cytochrome *c* oxidase I (COI) gene sequencing and phylogenetic analysis. The nucleotide sequences of all the populations showed 100 % similarity and the phylogenetic analysis confirmed the genetic similarity of *A. gossypii* in Taiwan and Cameroon. Results of the screening trials showed that three accessions (VI033805, VI036213 and VI051114) were resistant to *A. gossypii*. The basis of resistance of the three okra accessions was elucidated by studying their biochemical and biophysical properties. There was no significant difference between the susceptible and resistant okra accessions in terms of leaf tannins, free amino acids, total sugars and total phenols. Only total nitrogen was significantly different between the susceptible and the two resistant okra accessions with the lowest aphid infestation (VI033805 and VI036213). Thus, higher leaf nitrogen content seems to favour the aphid infestation on okra. For physical parameters, there was no significant difference among the accessions in trichome density of bottom and middle leaves, and leaf toughness. However, trichome density in the younger leaves of resistant VI033805 was significantly higher than susceptible VI057245. Studies on settling behaviour showed that aphids did not discriminate between the susceptible and resistant okra accessions for oviposition and feeding 72 h after release.

Key words: crop resistance, okra accessions, aphid populations, biophysical and biochemical basis.

INTRODUCTION

Okra (*Abelmoschus* spp.) is an important vegetable in tropical Asia and sub-Saharan Africa. Worldwide production of okra is estimated at 8.06 million tons annually. India is the world's leading okra producer (72 % of total production), followed by Nigeria (13 %). West Africa accounts for about 75 % of production in Africa (FAO 2011). West African okra (*Abelmoschus caillei*, also known as Guinean type) accounts for only 5 % of the total world production of okra (Siemonsma & Kouame 2004). In Cameroon, the two okra species, *A. caillei* and *A. esculentus* (common okra) combined represent the second most important vegetable in the market. Okra is cultivated mainly for immature pods consumed fresh or dried; added to soup, depending on the location. Okra is sometimes added to coffee or consumed as a coffee substitute. The mucilage in okra leaves and pods thickens soups and sauces (Nwangburuka 2010). Mucilage also has industrial applications as an adhesive and in the manufacture of candy. In medicine, mucilage is used as a plasma replacement or blood volume expander, and for cholesterol reduction (Markose & Peter 1990; Benchasri 2012). The pods contribute viscous fibre to the diet (Kendall & Jenkins 2004) and the viscosity eases consumption of hard foods (Schippers 2000). Increasing okra production can

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diversify vegetable production systems in sub-Saharan Africa and help improve diets (Hughes 2009).

The melon aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), is one of the major pests of okra, particularly in tropical and subtropical regions (Kersting *et al.* 1999), including Cameroon (Kekeunou *et al.* 2006). Aphids have a short life cycle but an extremely high reproductive rate. They reproduce throughout the year both parthenogenetically and sexually. Heavily infested okra plants commonly show distorted and stunted leaves and reduced fruit set (Wanja *et al*. 2001). Yield losses can be up to 57 % (Shannag *et al.* 2007) when aphid infestation is exceedingly higher (>1000 aphids per plant) (Mohamed-Ahmed 2000; Nderitu *et al.* 2008). This pest has also been reported on cotton, which is one of the major host plants of this pest in Cameroon (Ekukole 1990). The severity of aphid infestation has led to widespread use of chemical pesticides for control and okra ranks fourth among the vegetable crops that receive more chemical pesticides in Cameroon (Abang *et al*. 2013). Pests including aphids are becoming resistant to pesticides and *A. gossypii* has developed resistance to carbamates, organophosphates, pyrethroids and neonicotinoids (Wang *et al.* 2002; Andrew *et al.* 2006; Tabacian *et al*. 2011).

Resistant plant varieties form an important component in integrated pest management strategies. Some reports have confirmed the availability of aphid-resistant okra genotypes (Sumathi 2005; Anitha & Nandihalli 2009). However, most of these reports were based on a few local genotypes. For instance, only 15 local cultivars were screened in Tamil Nadu, India by Sumathi (2005). Cultivars such as Varsha Uphar and Arka Anamika were found to be moderately resistant. Anitha & Nandihalli (2009) evaluated only seven cultivated okra lines (mostly hybrids) for their resistance to aphid. No studies were carried out to elucidate the basis of resistance. Hence there has been no concerted effort to identify aphid-resistant genotypes from a broader gene pool across the globe. The AVRDC Genebank, the world's largest public vegetable germplasm collection, conserves more than 900 accessions of *Abelmoschus* spp., which offers broader gene pool required for a robust screening for resistance to aphid infestation. Hence the current study was carried out to identify aphid-resistant okra accession(s) from this broad gene pool.

Plant morphological characters and primary as well as secondary metabolites play a vital role in imparting resistance to various insect pests including aphids. Trichomes have either adverse (Zarpas *et al.* 2006) 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 also play a vital role in enhancing 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). On a virus aphid transmission (*Vat*)-resistant melon plant, *A. gossypii* seldom reached the phloem, or stopped feeding in phloem when reached (Chen *et al*. 1996; Klingler *et al.* 1998), and then starved. The levels of certain components such as amino acids and sugars in a plant may partially determine the likelihood of *A. gossypii* infestation (Deguine & Hau 2001).

Most aphid resistance genes identified to date are restricted in their effectiveness to single aphid species, or even to particular biotypes. For example, resistance in melon plants appears to be restricted to *A. gossypii* only (Klingler *et al.* 1998). *Medicago truncatula* cultivars that are resistant to *Acyrthosiphon kondoi* and the spotted alfalfa aphid *Therioaphis trifolii* f. *maculate* did not affect the infestation by *M. persicae* or cowpea aphid (*Aphis craccivora*) (Gao *et al.* 2007). Aphid resistance in plants appears to be species-specific; hence, it is important to confirm the species and/or biotypes of the target aphid population when selecting resistant cultivars.

The objectives of this study were to (i) investigate the genetic diversity of *A. gossypii* that occurs in Cameroon and Taiwan, (ii) identify the *Abelmoschus* spp. accessions that are resistant to *A. gossypii*, and (iii) characterize the biophysical and biochemical bases of aphid resistance that lead to antixenosis and antibiosis.

MATERIAL AND METHODS

Genetic similarity of A. gossypii *from study locations*

Four populations of *A. gossypii* were collected from okra and hibiscus (*Hibiscus rosa-sinensis* L.) in high-altitude savanna (06°1′N 10°00′E and 1260 m above sea level) and warm and humid forests (03°52′N 11°28′E and ~750 m above sea level) in

Cameroon. A fifth population was collected from okra in Shanhua, Taiwan (23°08′29″N 120°19′15″E). A reference population collected from okra in Salem, India (11°59′N 78°01′E) was also used. All the samples were collected under field conditions. At least 100 insects per population were collected from different fields in a location during the first quarter of 2012. The insects were preserved in glass vials containing 95 % ethanol for laboratory use. A total of 60 collected insects (10 per population) were used for cytochrome *c* oxidase subunit I (COI) sequencing and phylogenetic analysis. An outgroup (*A. craccivora*) sequence was obtained from the NCBI GenBank (HQ528252).

DNA was extracted from 10 individuals for each population. The insects were placed in a 1.5 ml Eppendorf tube containing 50 μl of Universal All™ extraction buffer (Yeastern Biotech, Taipei, Taiwan) and carefully crushed using a plastic pestle. The ground samples were centrifuged for 3 s and incubated in a water bath at 95 °C for 10 min. The solution was vortexed for 1 s and centrifuged for 2–3 s. The extracted DNA was diluted in a 1:10 ratio using deionized water and stored at –20 °C.

The primers Ago_CoxI_f2 (5′-CTTACCTGTAT TAGCTGGTGCTAT-3′) and Ago_CoxI_r2.1 (5′-GTTCTAATGGTGGAAGATTGTG-3′) were used to amplify the partial sequence of the COI gene of aphids from different locations. Polymerase chain reaction (PCR) was performed in a final reaction volume of 30 μl. The reaction components contain 11.4 μl of distilled water, 3 μl of 10X Super-Therm Gold Buffer, 2.4 μl of 2.5 mM dNTP mixture, 1.2 μl of 25 mM $MgCl_2$, 3 μl of 10 mg/ml BSA, 2.4 μl of forward and reverse primers, 0.6 μl of 2 U/μl Super-Therm Gold DNA Polymerase (Bertec Enterprise, Taipei, Taiwan), and 6 μl of aphid genomic DNA sample. The PCR programme used for amplifying aphid COI was an initial denaturation at 95 °C for 10 min, followed by 35 cycles made up of a denaturation step at 95 °C for 30 s, annealing at 60 °C for 45 s, extension at 72 °C for 1 min and final extension at 72 °C for 5 min. The PCR products were separated on a 2 % agarose gel and stained with ethidium bromide to visualize the amplified products under UV light. Direct sequencing of the PCR products was carried out (Genomics BioSci & Tech Co., Taiwan), and forward and reverse sequences were obtained using the corresponding PCR primers on both the DNA strands. The retrieved sequence data were aligned by the ClustalX2 program (Larkin *et al.* 2007) and subjected for subsequent phylogram construction using MEGA5 and using the Kimura two parameter distance model (Tamura *et al.* 2011).

Germplasm screening

Okra germplasm (*A. esculentus*) used in this study was obtained from the Genetic Resources and Seed Unit, AVRDC – The World Vegetable Centre, Taiwan. Four commercially available varieties in Cameroon were also included. Preliminary screening was conducted in unreplicated trials. Three preliminary screening trials, *viz.* spring (March to May) 2011 (88 accessions) and autumn (September to November) 2011 (68 accessions) in Shanhua, Taiwan, and November 2011 to February 2012 (112 accessions) in Nkolbisson, Yaoundé, Cameroon (03°51.79′N 11°27.71′E) were conducted. The trials in each country were carried out in one field in each season. The trials were maintained following customary cultural practices, and without pesticide application to control aphids or other sucking insects. The trials were exposed to the natural infestation of aphids and aphid populations were directly scored at weekly intervals starting from four weeks after transplanting in the field. Ten plants of each accession were randomly selected and scored using the following rating scale: 0 = no aphids present; 1 = 1 to 10 aphids per leaf; 2 = 11 to 100 aphids per leaf; 3 = 101 to 500 aphids per leaf; and 4 = >500 aphids per leaf. 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 & 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.

The selected resistant okra accessions with the known susceptible control from preliminary screening trials conducted in Cameroon and Taiwan in 2011 were screened in an advanced screening trial in Cameroon during March–June 2012. This advanced trial was conducted using a randomized block design with three replicates as a confirmatory screening. The crop management and screening methods were similar to the preliminary screening trials. Ten plants were randomly selected in each replicate to record the aphid population.

Biochemical analyses of resistance

The following biochemical parameters were estimated in okra leaves of selected resistant accessions (VI033805, VI036213 and VI051114) and the most susceptible accession (VI057245). The plants were grown in a controlled environment without insect infestations. Six leaves (two from the top, middle and bottom part of the plant) were collected for this study. Leaf samples were collected at the start of flowering and dried at 80 °C for 12–24 h. The dry samples were ground in an electric blender. For the analysis of phenols, fresh leaf samples were used with two leaves collected per plant stratum. The samples were replicated five times with two plants per replicate.

Analysis of tannins in leaves of aphid-resistant and susceptible accessions was conducted using the Catechin standard and acidified vanillin method (Broadhurst & Jones 1978). One gram of powder sample per replicate was used for analysis.

Total sugar content of leaves in aphid-resistant and susceptible accessions was spectrophotometrically determined using the 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.

Total amino acid content in the leaves of aphid-resistant and susceptible accessions was determined by the formaldehyde titration method (Sorensen 1907). Amino acids were extracted from a powder sample of 1 g per replicate.

The colorimetric method of Folin-Denis as described by Swain & Hillis (1959) was employed to determine the phenolic compounds in the leaves. The procedure consists of grinding 10 g fresh leaf samples per replicate.

Total leaf nitrogen content was determined following Kjeldahl's method as described by Bremner & Mulvaney (1982). The procedure consists of digesting 0.2 g of sample powder per replicate with 5 ml concentrated H_2SO_4 in a 50 ml Kjeldahl digestion flask.

Biophysical basis of resistance

The physical basis of resistance, *viz.* leaf trichome density (Bourland *et al.* 2003) and toughness, was examined in aphid-resistant and susceptible accessions. Three leaves, one each from the top, middle and bottom part 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 hairs was counted. Similarly, about 1 cm^2 leaf pieces were collected from either side of the mid-vein and mounted on a gram gauge to evaluate leaf toughness. 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 & Center (1997). Measurements of leaf toughness and trichome densities were recorded in five replicates with two plants per replicate for each accession.

The settling behaviour of aphids on aphid-resistant and susceptible accessions was observed. The second fully expanded leaf from the apex of two-week-old okra plants was collected. The cut ends were placed in moist cotton wool sealed with aluminum foil to keep the leaves fresh. The leaves of the four accessions were arranged in a circle and inserted midway under a circular disc of cardboard, with the adaxial surface facing upwards. About 100 aphids after 2 h of starvation were placed on the cardboard equidistant from each okra accession. The aphids and leaves were covered with black cloth to prevent light from affecting the aphids' behaviour. After 72 h, aphids were counted to evaluate their presence on each leaf and the accessions were rated as preferred or non-preferred by aphids. The experiment was replicated five times with two plants per replicate for each accession.

Statistical analysis

The AUIPC values for aphid population per leaf were subjected to a statistical analysis based on mean (m) and standard deviation (S.D.) (AVRDC, 1979), and categorized as follows: the accessions that had mean damage score (n) less than m – 2 S.D. were considered highly resistant; between m – 1 S.D. to m – 2 S.D. as resistant; between m to m – 1 S.D. as moderately resistant; between m to m + 1 S.D. as moderately susceptible; between m + 1 S.D. to m + 2 S.D. as susceptible, and more than m +2 S.D. as highly susceptible.

Data obtained from experiments on the bases of resistance were subjected to analysis of variance (ANOVA) with the Proc GLM procedure of SAS, version 9.1 (SAS Institute, Cary, NC, U.S.A.). Least significant difference (LSD) test was used to separate the means at 5 % significance level of probability. The choice test on the settling behav-

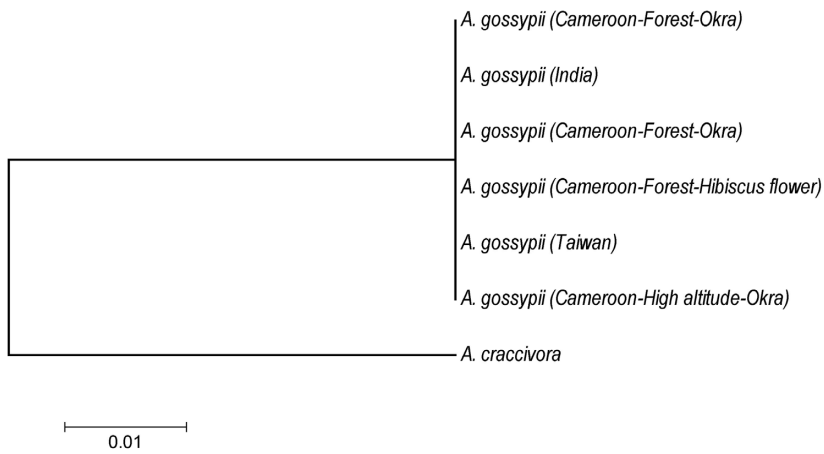

Fig. 1. Phylogenetic analysis between DNA of six populations of *Aphis gossypii* from Cameroon, India and Taiwan and that of *A. craccivora* retrieved from GenBank used as an outgroup.

iour of aphids was analysed using Friedman's test, followed by the appropriate non-parametric *post hoc* comparison.

RESULTS

Genetic diversity of aphid species

The primers used for sequencing produced a PCR product with approximate 900 base pairs. The sequences have been submitted in the NCBI GenBank and the accessions numbers are as follows: Cameroon [high-altitude savanna population (KF385392), farmer okra field in warm and humid forest (KF385393), *Hibiscus* plant in warm and humid forest (KF385394), on-station okra at warm and humid forest (KF385395)], Taiwan (KF385396) and India (KF385397). The nucleotide sequence comparison showed 100 % similarity between the populations as a result of sequence homogeneity (Fig. 1). The phylogenetic analysis also confirmed that the six aphid populations from Cameroon, India and Taiwan are genetically identical. *A. craccivora* was used as an outgroup and the nucleotide sequence comparison showed 93 % similarity with *A. gossypii*.

Okra accessions resistant to aphids

Out of the 88 accessions screened during spring 2011, eight accessions were rated as resistant to aphid infestation in Taiwan (Fig. 2). In a subsequent preliminary screening in autumn 2011, four of 68 accessions were rated as moderately resistant, and none of the accessions were rated as either resistant or highly resistant (Fig. 3). During the preliminary screening period in Cameroon from November 2011 to February 2012, 13 resistant accessions were identified (Fig. 4). Thus, a total of 25 okra accessions from three preliminary screening trials were selected for advanced screening. However, only 19 accessions (VI060740, VI060784, VI060787, VI060809, VI060810, VI060858 VI060313, VI058525, VI058521, VI058519, VI050960, VI050958, VI059164, VI056457, VI046559, VI051114, VI036213, VI033805 and Evodoula) were screened in the advanced screening trial; seed of the remaining six lines did not germinate.

Out of 19 accessions, three accessions (VI051114, VI036213 and VI033805) were rated as resistant to aphids (Table 1). These accessions are all *A. esculentus*, whereas in Cameroon *A. caillei* is the most cultivated species. *A. caillei* has triangular calyx segments and *A. esculentus* has spiny-shaped calyx segments. More than 20 accessions of *A. caillei* were screened during the present study, which included two commercial varieties (Gombo cafeier and Gombo paysan). In our results all *A. caillei* accessions screened were more susceptible to aphids in Cameroon. VI051114, locally known as *Utong,* was collected during 2002 from La Union province (16°45′07.7″N 120°28′22.6″E) in the Philippines. VI036213 was collected during 1991 from Rizal province (14°31′N 121°15′E), and VI033805 was collected during 1991 from Ilocos Sur province (17°20′N 120°30′E) in the Philippines. These three accessions originated from Luzon Island in the Philippines and are open pollinated types. VI036213 and VI033805 are old cultivars. All three cultivars have green leaves with red or purple veins. VI036213 has glabrous leaves, whereas the other two cultivars have intermediate

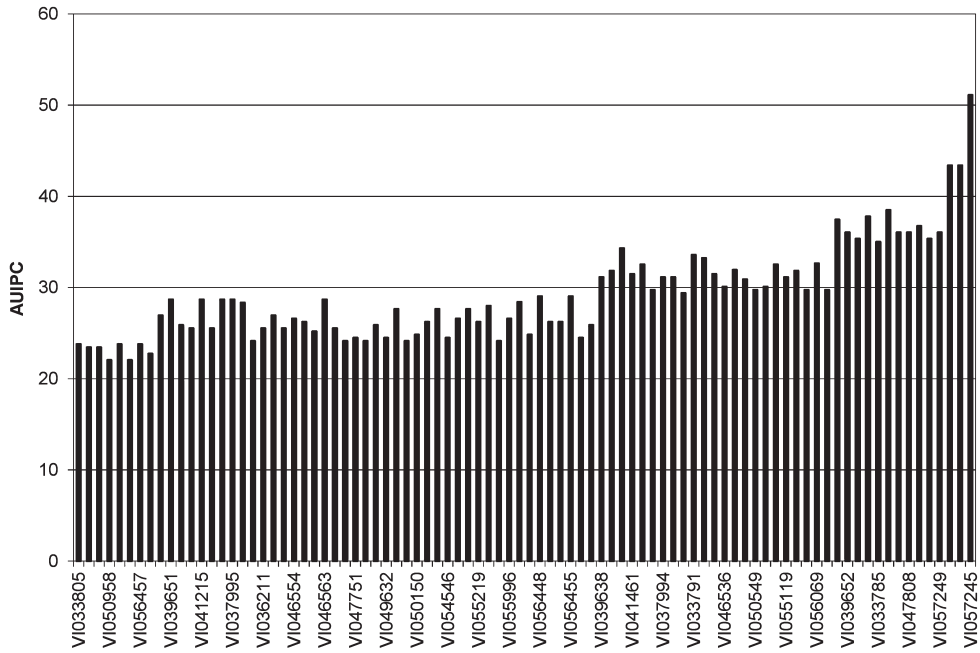

Fig. 2. Area under the infestation pressure curve (AUIPC) of okra accessions assessed for *Aphis gossypii* numbers in the field at Shanhua, Taiwan, spring 2011.

hairiness on the leaves. VI033805 and VI051114 bloomed earlier, in about 50 days; however, the time to flowering is more than 65 days in VI036213. VI033805 has relatively short (8 cm) but stout (3.33 cm diameter) pods, compared to VI051114 and VI036213, which have pods about 21 cm long and 2.3 cm in diameter. Thus, VI051114 and VI036213 have acceptable horticultural traits

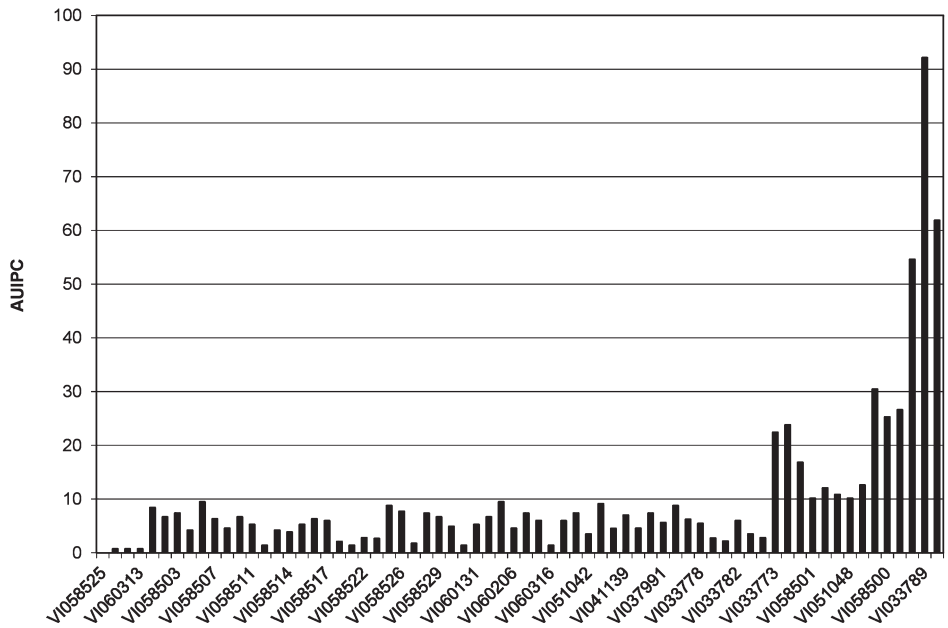

Fig. 3. AUIPC of okra accessions assessed for *Aphis gossypii* numbers in the field at Shanhua, Taiwan, autumn 2011.

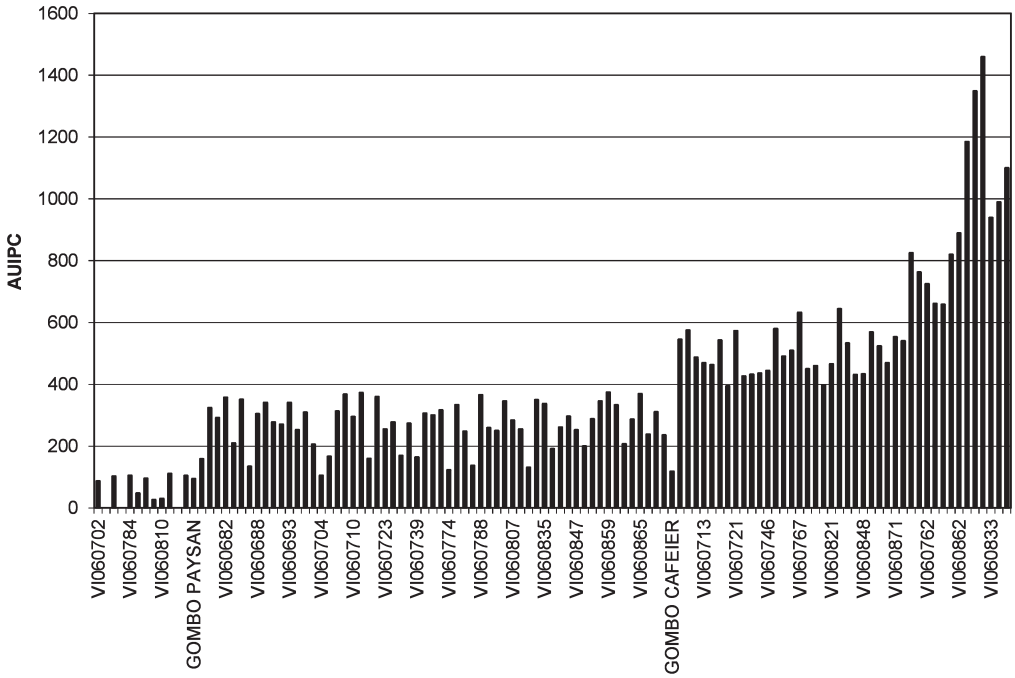

Fig. 4. AUIPC of okra accessions assessed for *Aphis gossypii* numbers in the field at Yaoundé, Cameroon, November 2011 to February 2012.

(except late flowering in VI036213) with appreciable aphid resistance. Although VI033805 has higher aphid resistance and early blooming, the pod length is too short.

Biochemical basis of resistance

There was no significant difference among the accessions in total sugar and tannin contents ($P = 0.55$ and $P = 0.37$, respectively) (Table 2). However, there were significant differences among the accessions for free amino acids ($P = 0.03$), total phenols ($P = 0.005$) and total nitrogen ($P = 0.0001$). The susceptible accession had higher amounts of total nitrogen and total phenols, which were on par with resistant accession VI051114 that had comparatively higher aphid infestation than VI036213 and VI033805 (Table 1).

Biophysical basis of resistance

There was no significant difference in leaf trichome density in leaves from middle ($P = 0.65$) and bottom ($P = 0.29$) strata among the accessions. However, the trichome density in the younger leaves of VI033805 (which had the lowest aphid infestation) was significantly higher than VI051114 ($P = 0.04$) (Table 3).

For leaf toughness, there was no significant difference among the accessions for the force needed to puncture the leaves from top ($P = 0.87$), middle ($P = 0.86$) and bottom ($P = 0.76$) strata (Table 4).

Settling behaviour

Studies on settling behaviour showed that aphids did not discriminate between susceptible and resistant okra accessions after 72 h of release ($P = 0.65$) (Table 5). However, the trend showed a higher aphid colonization on the susceptible accession.

DISCUSSION

Genetic studies on the populations of *A. gossypii* from Cameroon and Taiwan with an Indian reference population showed that *A. gossypii* is the most common aphid species on okra collected in Cameroon, Taiwan and India; no other species was identified during our study. An earlier study identified two biotypes (melon and cotton) of *A. gossypii* (Guldemond *et al.* 1994). Although the melon and cotton *A. gossypii* biotypes are morphologically indistinguishable, they have distinct host ranges. Studies have shown some differences in

Table 1. Area under the infestation pressure curve (AUIPC) of okra accessions assessed for *Aphis gossypii* numbers in the field at Yaoundé, Cameroon, March–June 2012.

Accessions	Origin	Total AUIPC	Resistance status
VI060313	Tanzania	572.0	Highly susceptible
VI050960	Zambia	421.8	Susceptible
VI058521	Unknown	363.0	Moderately susceptible
VI060740	Unknown	339.4	Moderately susceptible
VI058525	Unknown	335.5	Moderately susceptible
VI050958	Zambia	324.7	Moderately susceptible
VI060810	Turkey	320.6	Moderately susceptible
VI060784	USA	314.7	Moderately susceptible
VI059164	Unknown	300.6	Moderately susceptible
Evodoula	Cameroon	253.7	Moderately resistant
VI058519	Unknown	229.8	Moderately resistant
VI060787	USA	224.9	Moderately resistant
VI060809	Turkey	221.6	Moderately resistant
VI056457	Yugoslavia	207.8	Moderately resistant
VI060858	Mali	202.4	Moderately resistant
VI046559	Thailand	174.5	Moderately resistant
VI051114	Philippines	157.8	Resistant
VI036213	Philippines	134.7	Resistant
VI033805	Philippines	87.9	Resistant
Mean (*m*)		273.02	
Standard deviation (S.D.)		112.59	
m – 2 S.D.		47.84	
m – S.D.		160.43	
m + 2 S.D.		498.21	
m + S.D.		385.61	

host preference (Wang *et al.* 2004) and feeding behaviour (Gutierrez *et al.* 2008) between the melon and cotton biotypes. Crop resistance to *A. gossypii* also has been shown to be biotype-specific (Dogimont *et al.* 2008). There have been no reports about the occurrence of *A. gossypii* biotypes on okra. Our study showed that *A. gossypii* attacking okra in Cameroon, India and Taiwan are not genetically diverse. Hence, okra accession(s) with appreciable levels of aphid resistance in Taiwan may react the same way in Cameroon, unless environmental factors alter the resistance reactions.

The okra accessions/varieties screened in this

Table 2. Amount of total sugars, tannins, free amino acids, total phenols and total nitrogen in selected *Aphis gossypii*-resistant and susceptible okra accessions.

Accession	Resistance category	Total sugars (mg/g)	Tannins (mg/g)	Free amino acids (% DM*)	Total phenols (mg/100 g)	Total nitrogen (% DM)
VI057245	Susceptible	1.8 ± 0.07 a	0.7 ± 0.01 a	0.09 ± 0.006 ab	90.2 ± 1.53 a	2.75 ± 0.09 a
VI033805	Resistant	1.6 ± 0.01 a	0.7 ± 0.02 a	0.08 ± 0.002 b	70.4 ± 0.74 b	1.85 ± 0.01 b
VI051114	Resistant	1.6 ± 0.08 a	0.7 ± 0.02 a	0.10 ± 0.012 a	81.4 ± 0.49 a	2.43 ± 0.04 a
VI036213	Resistant	1.5 ± 0.05 a	0.7 ± 0.02 a	0.07 ± 0.004 b	78.2 ± 0.72 b	1.95 ± 0.01 b
F-value		0.74	1.1	3.85	9.8	15.05
Treatment and Error d.f		3, 12	3, 16	3, 12	3, 8	3, 16
P-value		0.55	0.37	0.03	0.005	0.0001

Means with the same letter in a column are not significantly different at $P < 0.05$ by LSD.
*DM = dry matter.

Table 3. Leaf trichome density (number/cm^2) in selected *Aphis gossypii*-resistant and susceptible okra accessions.

Accession	Resistance category	Bottom	Middle	Top
VI057245	Susceptible	4.94 ± 0.61 a	10.32 ± 0.78 a	33.52 ± 1.87 ab
VI036213	Resistant	6.98 ± 0.71 a	15.12 ± 0.83 a	46.62 ± 3.98 ab
VI033805	Resistant	4.66 ± 0.50 a	13.54 ± 1.95 a	59.04 ± 3.70 a
VI051114	Resistant	7.16 ± 0.34 a	13.06 ± 1.41 a	32.40 ± 3.17 b
$F_{(3,16)}$-value		1.38	0.56	3.66
P-value		0.29	0.65	0.04

Means with the same letter in a column are not significantly different at $P < 0.05$ by LSD.

Table 4. Leaf toughness (g) in selected *Aphis gossypii*-resistant and susceptible okra accessions.

Accession	Resistance category	Bottom	Middle	Top
VI057245	Susceptible	22.32 ± 0.98 a	22.22 ± 0.72 a	22.02 ± 0.65 a
VI036213	Resistant	21.02 ± 0.71 a	21.36 ± 0.58 a	20.92 ± 0.82 a
VI033805	Resistant	20.14 ± 0.34 a	20.52 ± 1.00 a	20.56 ± 0.59 a
VI051114	Resistant	19.92 ± 1.19 a	20.42 ± 0.93 a	20.46 ± 0.83 a
$F_{(3,16\ df)}$-value		0.40	0.26	0.24
P-value		0.76	0.86	0.87

Means with the same letter in a column are not significantly different at $P < 0.05$ by LSD.
Values represent the force (g) needed to puncture the leaf.

study ranged from resistant to highly susceptible to aphid infestation. The diversity of sources and number of accessions and varieties used increased the chances of obtaining sources of resistance to aphids. Three okra accessions were identified as resistant to *A. gossypii* after screening about 270 accessions. Hence, these *A. esculentus* aphid-resistant accessions could be used for cultivation and in okra breeding programmes for aphid resistance.

Sugars are necessary for the normal growth and development of insects, and their concentration in host plants is positively correlated with feeding behaviour of insects. However, no correlation between total sugar concentration of okra leaves and field infestation by *A. gossypii* was observed in the current study. In contrast, Deguine & Hau (2001) observed that the sugar content was twice as high in *A. gossypii*-susceptible cotton leaves. However the effect of reducing sugars was less significant in leafhopper-resistant okra varieties (Singh & Agarwal 1988). Similarly, the sugar content did not influence the thrips population in onion (Saxena 1970). It was thus not a surprise to find that the sugar concentration of okra leaves did not influence *A. gossypii* feeding behaviour.

Table 5. Settling behaviour of *Aphis gossypii* on selected aphid-resistant and susceptible okra accessions.

Accession	Resistance category	Mean number of aphids per leaf after 72 h
VI057245	Susceptible	26.67 ± 8.2 a
VI036213	Resistant	17.00 ± 5.8 a
VI033805	Resistant	15.83 ± 5.9 a
VI051114	Resistant	15.00 ± 8.5 a
F_R-value		4.20
χ^2 3d.f.		7.815
P-value		0.2407

Means with the same letter in a column are not significantly different.

Plant nutrients such as carbohydrates and proteins (amino acids) are also important for the development of *A. gossypii* (Slosser *et al.* 1989). In our study, two resistant okra accessions (VI033805 and VI036213) with low aphid infestation had significantly lower total nitrogen and free amino acids. This corroborates results that the suitability of hosts to aphids is positively correlated to the nitrogen concentration, the content of free amino acids, or the ratio of amino acids to sugars in plants as demonstrated by Deguine & Hau (2001). However, many authors have shown contradictory

results. For instance, leaf nitrogen and organic compounds in okra did not have any effects on *A. gossypii* populations (Leite *et al.* 2007). According to Lu *et al.* (2009), total nitrogen and amino acids in cotton were not associated with resistance to *A. gossypii*. Since the total nitrogen content was significantly different between the susceptible and the two resistant okra accessions with lowest aphid infestation (VI033805 and VI036213), it could be concluded that the leaf nitrogen content has a positive effect on the host selection process of *A. gossypii*.

The tannin content did not vary among the resistant and susceptible okra accessions. Tannins are believed to offer protection against phytophagous insects by reducing digestibility, but the defensive effects of tannins cannot be generalized. The average probing duration and the total probing time by *A. gossypii* fed on an artificial diet containing tannic acids were significantly reduced (Ma *et al.* 2005). But Lu *et al*. (2009) found that tannin content was negatively correlated with *A. gossypii* resistance in cotton. Zucker (1982) found an inverse correlation for the effect of total phenols in *Populus anqustifolia* to a galling aphid, *Pemphigus betae*. Similarly, this study revealed that total phenols were lower in some resistant accessions.

The role of total nitrogen and free amino acids, tannins and total phenols in imparting resistance to *A. gossypii* in selected okra accessions in our study is inconclusive. One possible reason is that the phytochemical analysis was carried out using plant samples collected at the beginning of flowering. Plants of different ages may vary in the quantities of nutrients and toxins they contain; young okra leaves may have lower amounts of secondary compounds in the early stages of plant growth, which has been confirmed in oak (Feeny 1976), cotton (Zummo *et al*. 1984), and other plants. Hence, the phytochemical analysis in selected resistant and susceptible okra accessions should be carried out at different plant growth stages with- and without aphid infestation, in the future.

The trichome density on leaves from the middle and bottom strata did not vary significantly among the okra accessions. However, the trichome density in the younger leaves of VI033805, which had the lowest aphid infestation, was significantly higher. This may be due to the fact that younger okra leaves have more trichomes. In the field, aphids would have the choice to colonize those leaves which have lower trichome density thus avoiding the need of attacking the younger ones. A higher density of non-glandular trichomes has been observed in the apical part than in the middle and bottom parts of okra (Leite *et al.* 2007). It was also found that the leaf trichome density in okra affects *A. gossypii* (Leite *et al.* 2007). Since the trichome density in the younger leaves of the least infested okra accession was significantly higher, it could be suggested that the physical effects of pubescence may only partly influence the feeding preference of *A. gossypii* in okra, since the susceptible accession did not show any significant difference. In the current study, leaf thickness did not play any significant role among the selected okra accessions in imparting resistance to *A. gossypii*. However, leaf toughness should be measured at different stages of plant growth because Scriber & Feeny (1979) showed that leaf toughness was involved in making the leaves progressively less suitable as they age. The settling behaviour study revealed that *A. gossypii* did not discriminate between the resistant and susceptible okra accessions, possibly because the trichomes, have little or no effect on the aphid settling, which was demonstrated by several earlier studies (Sarria *et al.* 2010).

CONCLUSIONS

The *A. gossypii* populations collected from okra in Taiwan and Cameroon did not differ genetically. Out of 268 accessions, only three *A. gossypii* resistant okra accessions (VI033805, VI036213 and VI051114) were identified. The leaf tannins, free amino acids, total sugars and total phenols did not play any significant role in imparting resistance. Only total nitrogen content seems to increase the attractiveness of the crop to *A. gossypii*. For physical parameters, trichome density of bottom and middle leaves and leaf toughness did not have any effect on *A. gossypii* infestation among the okra accessions. However, the trichome density in the younger leaves of VI033805, which had the lowest aphid infestation, was significantly higher compared with susceptible VI057245. Further studies on settling behaviour showed that aphids did not discriminate between the susceptible and resistant okra accessions for feeding after 72 h of release.

Since the selected aphid-resistant okra accessions have good horticultural traits, they can be introduced for cultivation in Cameroon, and also be used in okra breeding programmes for aphid resistance. The breeders should use okra acces-

sions with appreciable levels of aphid resistance to avoid serious infestations in improved varieties.

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