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Molecular study of the population structure and geographic distribution of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in the North East Arid Zone of Nigeria

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This study was conducted to determine the population structure and geographic distribution of *Bemisia tabaci* (Whitefly) in north-east Nigeria, West Africa. Two localities each were selected from three States of North-eastern Nigeria for sample collections and determination of population structure and distribution. The localities include Toro and Wuro wasse in Bauchi State, College of Agric farm center (COAG) and Tudun wada in Borno State and Dadin kowa and Kwadon in Gombe State. Population genetic variability was performed on a total of 240 whiteflies using Polymerase Chain Reaction (PCR) and microsatellite DNA analysis on *mtCOI* as target genes using BEM12, BT-e49 and BT-b103 primers. The results of genetic characterization based on PCR and *mtCOI* nucleotide sequence identity revealed a clear geographic distribution of three biotypes B, Q and unknown. Comparison of amplified DNA size (bp) and mitochondrial cytochrome oxidase I subunit (*mtCOI*) sequence revealed a similarity of 97.2–99.5% of B and Q biotypes found in this study with others elsewhere while the unknown biotype probably heterozygote between Q and B were few in all locations. The average number of alleles (N_A) per locus was estimated to be 1.66. The average N_A ranged from 1.672 alleles (Toro) to a maximum of 2.116 alleles (Dadin kowa). The observed heterozygosity (H_O) varied among populations with a mean of 0.832. The inter-population variation index (F_{ST}) = 0.34.

Key words: *Bemisia tabaci,* biotypes, distribution, microsatellite markers, mitochondrial, Cytochrome oxidase I subunit.

INTRODUCTION

Bemisia tabaci is known for its genetic diversity, which is expressed in a complex of biotypes (Brown et al., 1995; Perring, 2001; DeBarro et al., 2005) or, as recently suggested, a complex of distinct cryptic species (Xu et al., 2010; DeBarro et al., 2011). The biotypes are largely differentiated based on biochemical and molecular polymorphism, and differ in their characteristics such as host plant range, the capacity to cause plant disorder, attraction by natural enemies, expression of resistance and plant virus transmission capabilities (Bedford et al., 1994; Brown et al., 1995; Sanchez et al., 1999; Perring, 2001; Horowitz et al., 2005). Recent reports have suggested that the floral composition of bacterial symbionts might be specific to certain biotypes (Gottlieb et al., 2006; Chiel et al., 2007) and might confer upon them resistance to insecticides (Kontsedalov et al., 2008). The most widespread biotype, B, was identified in the late 1980s (Costa et al., 1993) following extensive outbreaks of *B. tabaci* in Southwestern USA, and has a wide distribution. An additional common biotype Q, which probably originated in the Iberian Peninsula (Guirao et al., 1997), has since spread globally (Horowitz et al., 2003; Boykin et al., 2007; Chu et al., 2010). However, studies relating to the species complex distribution are lacking in the arid zone ecology of the North-Eastern Nigeria and therefore the aim of this study was to determine population structure and geographic distribution of *B. tabaci* in different locations in the

Northeast Nigeria.

MATERIALS AND METHODS

Study area

The study was conducted in three States of the Sudano-Sahelian ecological zone of Nigeria *viz:* Bauchi, Borno and Gombe States. These three States share some similar agro-ecology, which is characterized by unimodal rainfall ranging between 12 and 1200 mm annually, temperature ranges between 18.4 and 34°C, altitude of 600–1800 m (http://www.infonet-biovision.org/default/ct/690/agrozone). Vegetation is mainly Sudano-savannah grass land making the region suitable for the cultivation of various crop varieties including vegetables, bulbs, grain and tree crops, and also suitable for high rate of multiplication and infestation of crops by insect pests. In each State, two major areas of farming activities were selected *viz:* Wuro wasse and Toro (Bauchi); Tudun wada and COAG farms (Borno); and Kwadon and Dadin kowa (Gombe).

Bauchi is the Capital city of Bauchi State and is located in the Sudan-Savannah region of north-east Nigeria at Latitude 10°18' N and Longitude 9°50' E. Gombe on the other hand is the capital city of Gombe State and is located at Latitude 10°17' N and Longitude 11°10' E while Maiduguri is the Capital of Borno State and is located in the Sahel Savannah region of North-East Nigeria at Latitude 11°05' N and Longitude 13°05' E.

The temperature for the three States varies with time and location of an area. In Bauchi for instance, the mean daily maximum temperatures range from 29.2°C in July and August to 37.6°C in March and April. The mean daily minimum ranges between 11.7°C in December and January to about 24.7°C in April and May. While in Gombe, temperature ranges between 22 and 34°C and has similar pattern of monthly distribution like Bauchi. In Maiduguri, the rainy season months are May to September/October with its peak in August. Humidity ranges from 28 to 46%. Monthly rainfall ranges from 0.0 mm in December-April, to about 343 mm in August. Onset of the rains varies but more often in April/May while they end virtually by October. The sunshine hours range from about 5.1 h in July-September to about 10.9-12 h in the remaining months (http://www.infonet-biovision.org/default/ct/690/agrozones).

Field collection of Whitefly for genetic characterization

B. tabaci was sampled randomly from cotton (*Gossypium hirsutum)* and tomato (*Lycopersicon esculentus*) plant hosts in six locations of vegetable production areas in three States using modified plastic vials by vacuuming plant foliage with a Makita® Cordless Vacuum (Model 4071D) and custom made battery operated suction sampler (Dittrich et al., 1990). Sampling for the whitefly was performed for 2 h for each sample day from 6:00-7:00 am and 5:00-6:00 pm on monthly basis for six months. In each sample location, two sunken beds 10 by 20 m were prepared side by side 100 m away from farmers planting fields to avoid being spread with insecticides during routine farm spray (Dittrich et al., 1990). The beds served as reservoir for the *B. tabacci* throughout the collection period. 100 seedlings each of tomato and cotton was planted on each bed and allowed to grow under natural rainfall sometimes supplemented by irrigation water to prevent drying out of leaves with no insecticide application but routine farm practice were observed. All adult samples collected were counted and recorded right in the field while the larvae on the sampled plants from Gombe and Bauchi States were taken to Department of Biological Sciences laboratory, Gombe State University, Gombe for identification while samples collected from Maiduguri were taken to the Department of Biological Sciences Laboratory, University of Maiduguri for identification under microscope using keys as described by Chaubey et al. (2010) and Bellows et al. (1994).

Whitefly samples collected alive on the field were kept in *Bemisia*-proof cages containing cotton and tomato plants seedlings at seven to eight true-leaf stages in polybags and kept in green house of Borno State Ministry of Agriculture at Center for Agricultural transformation field Maiduguri, to continue their normal reproduction processes. Within one week of being brought into the green house, 10 adult *B. tabaci* from each collection site were randomly selected and preserved in 3 ml micro centrifuge tubes containing silica gel and stored in the laboratory at the Department of Biological Sciences, University of Maiduguri until used for genetic characterization of population biotypes.

Genetic variability and identification of biotypes using molecular techniques

Biotype determinations and genetic variability and distribution were performed on a total of 240 (20 samples per hosts plant that have been preserved in alcohol from each geographical locations from the three States) using published oligonucleotide primers BEM12, BT-e49 and BT-b103, and the polymerase chain reaction (PCR) to magnify a specific area of whitefly DNA in the mitochondrial cytochrome oxidase 1 units (COI) gene (Brown, 2001). The amplified DNA was then sequenced to detect biotype-specific differences in nucleotides as described by Li et al. (2003), Fontes et al. (2010), DeBarro et al. (2011) and Horowitz et al. (2014). All molecular analysis was partly carried out at Noguchi Memorial Institute for Medical Research, University of Ghana in collaboration with Sangon Technology Company, Shanghai, China following the manufacturers'

standard procedures.

Extraction of genomic DNA from whiteflies for biotype determination

The DNA of the whiteflies was extracted from individual whitefly using the modified form of Brown (2001). Individual whiteflies were placed on Para film with 15 µl DNAzol and 5 µl Polyacryl carriers and then homogenized in 100 µl Bender buffer using the rounded edge of a clean 1.5 ml micro centrifuge tube. The homogenate was incubated in a hot water bath at 65°C for 30 min, thereafter centrifuged at 14,000 rpm for 15 min and then transferred to a new 1.5 ml micro centrifuge tube containing 0.48 ml DNAzol and 2.5 µl of Proteinase K. The samples were kept at room temperature for 30 min before precipitation of DNA with 0.25 ml 100% ethanol. After centrifuging the samples at 14,000 rpm for 15 min, supernatant was recovered into a fresh tube living in the DNA pellet, and the resulting DNA pellet was washed in 150 µl of 75% ethanol and centrifuged for 5 min at 13,000 rpm. Excess ethanol was poured off from tubes and the DNA pellet was allowed to air-dry. The pellet was dissolved in 20 µl Tris-EDTA + RNAse (50 µg/ml), and kept at 4°C overnight and thereafter kept at -20°C until used for PCR.

PCR amplification of mtCO1 DNA and sequencing

All of the analysed microsatellite loci were amplified by PCR in a final volume of master mix of 24.5 µL prepared in an eppendorf tube. The composition of the master mix consist of 4 µl DNA (approximately 20.5 ng), 3.5 µl of the forward and reverse primers (0.2 µM), 0.5 µL dNTPs (0.15 mM), 3.5 µL 1X buffer (50 mM KCl, 100 mM Tris-HCl, pH 8.5), 1.75 µl $MgCl_2$ (4.0 mM), 0.5 U Taq DNA polymerase, and 10.75 µl autoclaved MilliQ water.

The amplifications were performed in a thermo cycler (Perkin Elmer, USA). The PCR protocol for each primer was: an initial denaturation step of DNA was conducted for 15 min at 72°C for 8 cycles, followed by 30 cycles of denaturing at 94°C 30 s, followed by primer annealing at 60°C for 30 s and the final extension was run for 1 min at 72°C. Three sets of primers were used in this analysis which include BEM12, BT-e49 and BT-b103 that had been previously described by DeBarro et al. (2003), Tsagkarakou and Roditakis (2003) and Tsagkarakou et al. (2007). The reaction mix was then held at 4°C in a Perkin Elmer DNA thermal cycle machine for amplification.

Gel electrophoresis for analysis of PCR products

Five microlilitres of each PCR product were mixed with 1 µl of Orange G (5X) gel loading dye and was electrophoresed in a 2% agarose gel stained with 0.5% ethidium bromide to detect the presence of amplified DNA fragments. The gels were electrophoresed in 1X TAE buffer using a midi gel system (BIORAD, USA) at 100 volts for 1 h and photographed over UV trans-illuminator (UPC, USA) at short wavelength using Polaroid camera and type 667 films (Polaroid, USA). The PCR products obtained were compared with Published sequences for *B. tabaci* obtained from Gene Bank using the National Centre for Biotechnical Information. Bands were excised from agarose gel and purified for DNA cloning using a Qiagen gel purification kit (QIAGEN, Venlo, the Netherlands) following the manufacturers procedure before sequencing.

Data analysis

Tests of Hardy-Weinberg equilibrium (HWE) were estimated for each locus by F stat v.2.9 soft wire (Weir and Cockerham, 1984). The genetic diversity was estimated by Genetix V.4.05 (Belkhir et al., 2002).

RESULTS

Population structure and geographic distribution of *B. tabaci*

The results of genetic characterization based on PCR and gene sequencing revealed a clear geographic distribution of three biotypes which include biotypes B, Q and unknown (CV). The distribution status of these biotypes is presented in Table 1.

In Toro (Bauchi State), more than 70% of the population comprised of Biotype B (Table 1), which is significantly different from Q and unknown Biotypes ($P < 0.05$) and which recorded 20 and 6.7%, respectively. Similarly, in Wuro wasse farm in the same Bauchi State, B biotype was also found to be highest with percentage frequency of 53.3 followed by Q and the unknown with percentage frequency of 33.3 and 13.3, respectively. In COAG and Tudun wada farms in Borno State, it was a different scenario. Q biotype was the predominant population in these two locations with percentage frequency of 53.3 and 60.0, respectively. In Gombe State, the B biotypes predominated the population in both sample location and are at par between Kwadon and Dadin kowa with percentage frequency of 63.3 followed by Q biotypes with percentage frequency of 30.0 and 16.7, respectively for Kwadon and Dadin kowa. Comparison of amplified DNA size (bp) revealed a similarity of 97.2- 99.5% of B and Q biotypes found in this study with reference Africa genotypes while the unknown biotype probably heterozygote between Q and B were not restricted to a particular location.

Table 1. Percentage distribution of *B. tabaci* biotype base on PCR amplification from study locations.

Location	% Biotype		
	B	Q	CV
Toro	73.3	20.4	6.3
Wuro wasse	53.3	33.3	13.3
coag	36.7	53.3	10.0
Tudun wada	25.0	60.0	15.0
Dadin kowa	63.3	30.0	6.7
Kwadon	63.3	16.7	20.0
Mean	**52.48**	**35.62**	**11.88**

Table 2. Estimates of genetic diversity parameters in six *Bemisia tabaci* populations.

Population	*N*	N_A	H_E	H_O	*f*
Toro	26	1.672	0.608	1.042	-0.984
Wuro wasse	26	1.413	0.496	0.737	-0.736
Tudun wada	26	2.046	0.735	0.656	-0.833
COAG	26	1.497	0.684	1.081	-0.818
Kwadon	26	2.560	0.295	0.868	-0.775
Dadin kowa	26	2.116	0.398	0.598	-0.824
Mean	26	1.666	0.542	0.832	-0.807

N = number of individuals sample analyzed by electrophoresis; N_A = average number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity under Hardy-Weinberg equilibrium; *f* = fixation index.

Table 3. Estimates of frequencies of exclusive alleles obtained in microsatellite loci in six *Bemisia tabaci* populations.

Population	Host plant	Loci	Allele (bp)	Frequency (%)
Toro	tomato	1.1	55	8.33
Wuro wasse	cotten	1.1	56	8.33
Tudun wada	tomato	2.3	75	98.75
COAG	cotten	2.3	108	98.75
Kwadon	tomato	37	129	50.00
Dadin kowa	cotten	37	130	50.00

Genetic variation based on gene sequencing

50 alleles were found in the 13 polymorphic loci. The average number of alleles (N_A) per locus was estimated to be 1.66 and the average expected heterozygosity under Hardy-Weinberg equilibrium (H_E) was 0.542. The average N_A ranged from 1.672 alleles (Toro) to a maximum of 2.116 alleles (Dadin kowa) (Table 2). The observed heterozygosity (H_O) varied among populations, ranging from 0.737 (Wuro wasse) to 1.042 (Toro), with a mean of 0.832. In contrast, the smallest H_E was obtained in the Kwadon 0.295 population, while the COAG population had the highest value for this parameter 0.684.

After correcting the significance level with the Bonferroni correction ($P \leq 0.05$), it was observed that the majority of the populations were in HWE for most loci. However for some few loci, a small number of exclusive alleles were observed in certain populations. The frequencies of these alleles are shown in Table 3. The deviations from the HWE might be related to the small sample sizes of some populations.

Genetic structure

The species rate of fixation (F_{IS}) estimated from 13 SSR loci was negative and significant, indicating an excess of heterozygotes. The inter-population variation index was high (F_{ST} = 0.34), indicating that the populations differed

Table 4. F-statistic estimates in 6 natural *B. tabaci* populations gene structure.

Parameter	*f* (*FIS*)	*f* (*FIT*)	θ (F_{ST})
Estimate	-0.7621	-0.2538	0.4822
Upper limit (95%CI)	-0.6342	-0.0598	0.44480
Lower limit (95%CI)	-0.8856	-0.4704	0.21509

95%CI = confidence interval at 95% probability

Table 5. Nei's genetic distances calculated among the *Bemisia tabaci* populations.

Population	Toro	Wuro wasse	Tudun wada	COAG	Kwadon	Dadin kowa
Toro	**0.000**	0.179	0.294	0.363	0.197	0.310
Wuro wasse	0.213	**0.000**	0.292	0.412	0.219	0.198
Tudun wada	0.889	0.739	**0.000**	0.255	0.286	0.108
COAG	0.354	0.608	0.506	**0.000**	0.254	0.264
Kwadon	0.409	0.363	0.306	0.399	**0.000**	0.173
Dadin kowa	0.240	0.276	0.399	0.211	0.056	**0.000**

Values below the diagonal are the genetic distances while the values above are the F_{ST} values calculated pairwise.

from one another, as evidenced by the large number of clusters detected. These estimates were relatively consistent and significantly different from zero (Table 4). These differences might be primarily related to the adaptation of the insects to their specific host plants and geographic isolation. The Nei genetic distances calculated among the populations ranged from 0.000 (diagonal) to 0.889 (Table 5).

DISCUSSION

The characterization of *B. tabaci* complex population by microsatellite DNA analysis revealed that the distribution of the alleles among populations was not uniform as evidenced by high differentiation index (F_{ST} = 0.482) compared to F_{ST} = 0.34 (Moya et al., 2001) and F_{ST} = 0.32 obtained by Delatte et al. *(*2006) suggesting that the population were not the same among the six studied locations. This finding agrees with the results of previous publications (De Barro et al., 2003; Tsagkarakou and Roditakis, 2003; Tsagkarakou et al., 2007; Gauthier et al., 2008) who also found a similar genetic variation when tested with various primers. However, a high level of genetic polymorphism was observed in the present study, compared to previous study (Simón et al., 2007; Dalmon et al., 2008; *do*Valle et al., 2011). The variation observed could be attributed to small geographic range where gene flow range has a short distance compared to above authors who analysed the polymorphisms in different population collected from a larger geographic range compared to the present study. However, for a population to have no evidence of a division of gene pool, the estimate of inter-population genetics differentiation (F_{ST}) ought to be <0.087 (Lehmann et al., 1997).

The average N_A of 1.33 found in this study seemed to be significantly less compared to some previous studies (Lehmann et al., 1997) who reported to have found 2.37. Similarly, the average H_E of 0.519 found in this study was higher compared to that found in a similar study (Delatte et al., 2006) who reported 0.604. The attributed reasons for this variation cannot be explained in this report. However, it could be that cryptic species that form the biotypes population consist of some subclade population that coexist; this need to be determined.

From a total of 240 samples of adult whitefly, all six populations were found to be heteromogeneous. The F_{IS} estimated from 13 SSR loci was negative (-0.762) and significant, indicating an excess of heterozygotes. This result contradicted the results of a previous study (Evanno et al., 2005), which showed a positive value (0.18), indicating the occurrence of intra-population endogamy. However, the previous study used different populations and different microsatellite loci, which might explain this discrepancy.

The F_{ST} in this study was high (F_{ST} = 0.482), indicating that the populations differed from one another, as evidenced by the large number of clusters detected by the Bayesian analysis. These estimates were relatively consistent and significantly different from zero. These differences might be primarily related to the adaptation of the insects to the host plants and geographic location. Previous studies have confirmed that specific populations could be reproductively isolated because of copulation and post-copulation barriers (Wang et al., 2010; Xu et al., 2010; Sun et al., 2011).

The high F_{ST} value 0.482 (Table 4) observed in the current study was inconsistent with the F_{ST} values documented in the published literature using random amplified polymorphic DNA markers (Moya et al., 2001; F_{ST} = 0.34), and those obtained by Delatte et al. (2006) (F_{ST} = 0.32) using microsatellite markers. Another interesting observation noticed in this study is the high number of null alleles among population as evidenced by allele amplification at different molecular ladder using the same primers expected to amplify at their expected band size. This cannot be explained clearly in this study; however, null alleles could be due to mutation in the region complementing with one or two of the oligonucleotide primers (Lehmann et al., 1997), and whether finding those more in these populations are evidence of heterozygote population or other species complex cannot be concluded from this study. Moreover, the occurrence of null alleles presents a common complication in the interpretation of microsatellite data (Pemberton et al., 1995; Lehmann et al., 1996).

Conclusion

This study has shown that the population structure of *B. tabaci* is diversed based on PCR analysis. The DNA amplification pattern observed showed that at least two distinct biotypes exist among the populations collected within the arid ecological zone of North-eastern Nigeria. These differences may be influencing reproductive potentials, the virus vectoring capabilities and also their resistance to insecticides used in their control, which needs to be investigated.

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