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Identification of Whitefly (*Bemicia tabaci* Genn.) Biotypes and Associated Bacterial Symbionts in Oman

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ABSTRACT

Oman is located on southeast corner of the Arabian Peninsula and the whitefly (*Bemicia tabaci* Genn) is a predominant pest responsible for vectoring begomoviruses. Whiteflies were collected during 2011-2012 from various crops grown in different regions of Oman for biotype and secondary symbiont identification. Polymerase Chain Reaction (PCR) amplification with specific primers was used for *B. tabaci* biotypes and secondary symbionts identification. Only biotype B of *B. tabaci* was identified from whitefly population collected from various crops in different regions of Oman. All B biotypes were found to be infected with the secondary symbiont, *Hamiltonella*. The presence of only biotype B strongly suggests that it is a well-adapted pest to a variety of crops grown in different geographical regions of Oman.

Key words: *Bemicia tabaci*, biotype, Oman, secondary symbiont, primary symbiont

INTRODUCTION

The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a polyphagous pest that feeds on a wide variety of crops including vegetables, ornamentals (Oliveira *et al*., 2001; Jones, 2003; Ma *et al*., 2004). Its broad host range has lead many studies aimed at understanding the preference of whitefly selection as well as the effect of the host on the whitefly fitness (Van Lenteren and Noldus, 1990; Gerling and Mayer, 1996). *B. tabaci* is complex cryptic species, displaying high degree of variability in their biology and genetics. It consists of at least 30 morphologically indistinguishable species, 11 of them were placed in a high well-defined group according to biochemical and molecular markers (Dinsdale *et al*., 2010; Hu *et al*., 2011; Alemandri *et al*., 2012). Previous terminologies considered *B. tabaci* as a collection of biotypes that consists of 24 biotypes which differ genetically and in host range, insecticide resistance and plant virus transmission (Brown, 1994; Perring, 2001). The two widespread polyphagous biotypes of *B. tabaci* are the Middle East Asia Minor 1 (MEAM1, known as B biotypes) and Mediterranean (MED, known as Q biotype) (Skaljac *et al*., 2013). The B and Q biotypes, are globally spread due to international trade (De Barro and Ahmed, 2011; De Barro *et al*., 2005). B and Q biotypes of *B. tabaci*, rarely interbreed despite having narrow geographical origin. The first invasion by biotype B replacing biotype A in USA was recorded in 1980s (Costa *et al*., 1994; Perring *et al*., 1993). In India biotype B was first reported in 1999 in the Kolar district of Karnataka State which spread rapidly to other states in south India in a span of 2 years period (Rekha *et al*., 2005). *B. tabaci* biotypes are morphologically indistinguishable and their identification is solely dependent

on molecular techniques. However, De Barro *et al.* (2005) grouped *B. tabaci* populations into six geographical races based on COI and ITS1 gene sequences but the biotypes are still used as common nomenclature.

B. tabaci harbors the obligatory primary endosymbiont, *Portiera aleyrodidarum* which is important for its survival and development. In addition, *B. tabaci* harbors other facultative Secondary Symbionts (SS) with unknown function (Thao and Baumann, 2004; Baumann, 2005; Gottlieb *et al.*, 2006; Chiel *et al.*, 2007). The secondary symbionts associated with *B. tabaci* are *Arsenophonus, Hamiltonella, Wolbachia, Cardinium, Fritschea* and *Rickettsia* (Baumann, 2005; Everett *et al.*, 2005; Gottlieb *et al.*, 2006). However, only *Hamiltonella* and *Rickettsia* have so far been reported associating with *B. tabaci* population in the Middle East (Gottlieb *et al.*, 2006; Chiel *et al.*, 2007).

Although heavy infestations of whiteflies are observed in all regions of Oman on various crops, biotype identity so far is not determined. This study was undertaken to identify whitefly biotypes and associated secondary symbionts from different regions of Oman infesting different crops. This is the first report from Oman on identification of *B. tabaci* biotype and secondary symbiont.

MATERIALS AND METHODS

Whiteflies were collected from different regions/Governorates of Oman during 2011-2012 and the location from which samples were collected is shown in Fig. 1. Adult whiteflies were collected from different crops, tomato, pepper, cucumber, eggplant, squash and petunia (Table 2) and preserved in 95% ethanol until analyzed.

Total nucleic acids were extracted from individual whitefly using lysis buffer (Frohlich *et al.*, 1999). A single whitefly was macerated into 0.5 mL Eppendorf tube with lysis buffer (5 mM Tris-HCl, pH 8.0, 0.5 mM of EDTA, 0.5% Nonidet P-40 and 1 mg mL^{-1} of proteinase K) using disposable sterile pestle. The mixture was incubated at 65°C for 15 min followed by 95°C for 10 min before a brief centrifugation (10000xg) to pellet the debris. The supernatant containing total nucleic acid was stored at -20°C until used.

To identify biotypes, total extracted nucleic acid was used as template for PCR in 25 μL reaction mixture (2 μL DNA template, 2.5 mM $MgCl_2$, 0.15 μM dNTP, 0.75 U Taq polymerase and 0.6 μM of each primer Bem23F [5'-CGGAGCTTGCGCCTTAGTC-3'] and Bem23R [5'-CGGCTTTATCATAGCTCTCGT-3']) as described previously "(De Barro *et al.*, 2003)". The PCR primers Bem23F/Bem23R amplify a fragment of 200 bp from the B biotype and 400 bp bp for Q biotype of mtCOI gene of whitefly (Table 1). The PCR reaction mix was subjected to the 35 cycle of the thermal cycler program (denaturation 2 min at 95°C followed by 1 min at 94°C, annealing at 55°C for 1 min, extension 72°C for 1 and 5 min extension at 72°C for final cycle). The PCR products were separated on 0.8% agarose gel stained with ethidium bromide and photographed on Syngene DNA documentation system.

To determine secondary symbionts associated with the whitefly populations collected, the total nucleic acids extracted were used as template for PCR. The reaction mixture of 25 μL (2 μL template DNA lysate, 0.2 mM dNTP, 1U Taq DNA polymerase and 10 pmol of each primers) was incubated in ABI Veriti 9600 thermal cycler at 65°C for 15 min followed by 10 min at 95°C. The presence of secondary symbionts, *Rickettsia, Hamiltonella, Wolbachia, Cardinium, Arsenophonus* and *Fritschea* in the extracted nucleic acid lysate was determined using genus specific primers designed to amplify a fragment of 16S/23S ribosomal gene (Table 1). PCR amplicons were visualized on 1.2%

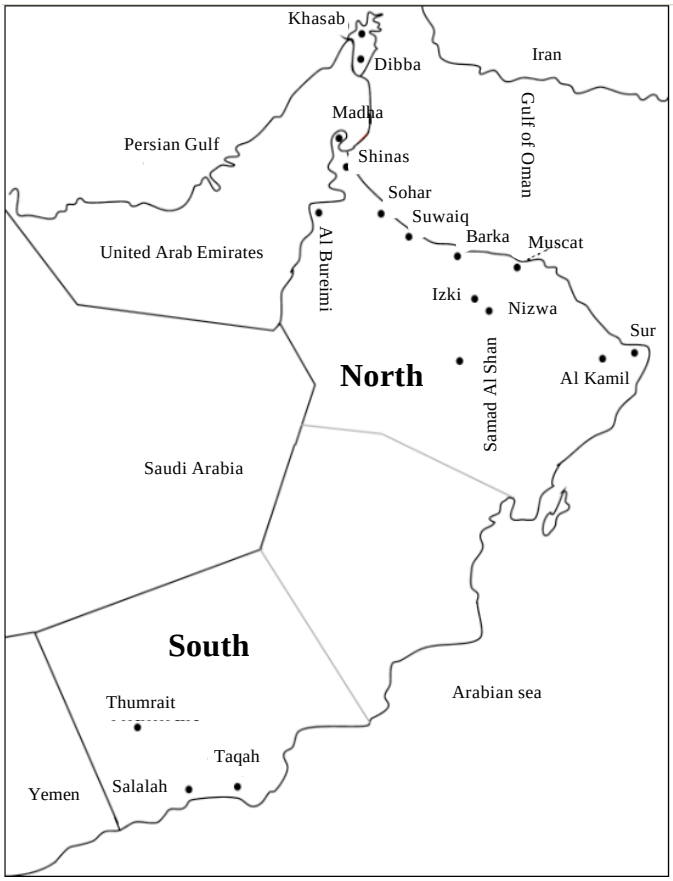

Fig. 1: Geographic map of Oman showing the places from where whitefly (*Bemisia tabaci* Genn.) samples were collected

Table 1: List of biotype specific and genus specific primers for different secondary symbionts for polymerase chain reaction

Biotype/symbiont	Primer name	Primer sequence (5'to 3')	Annealing temp/amplicon size	References
B. tabaci biotype	Bem23F	CGGAGCTTGCGCCTTAGTC	55°C	De Barro *et al.* (2003)
	Bem23R	CGGCTTTATCATAGCTCTCGT	200 bp B biotype	
			400 bp Q biotype	
Rickettsia	RbF	GCTCAGAACGAACGCTATC	59 0C/900 bp	Gottlieb *et al.* (2006)
	RbR	GAAGGAAAGCATCTCTGC		
Hamiltonella	92F	TGAGTAAAGTCTGGGAATCTGG	62 0C/700 bp	Zchori-Fein and Brown (2002)
	HbR	AGTTCAAGACCGCAACCTC		
Arsenophonus	Ars23S-1	CGTTTGATGAATTCATAGTCAAA	59 0C/580 bp	Tao and Baumann (2004)
	Ars23S-2	GGTCCTCCAGTTAGTGTTACCCAAC		
Wolbachia	Wol16SF	RCGGGGGAAAAATTTATTGCT		
Wol16S		AGCTGTAATACAGAAAGTAAA	55 0C/650 bp	Chiel *et al.* (2007)
Cardinium	CFBF	GCGGTGTAAAATGAGCGTG	59 0C/500 bp	Weeks and Breeuwer (2003)
	CFBR	ACCTMTTCTTAACTCAAGCCT		
Chlamydia	U23F	GATGCCTTGGCATTGATAGGCGATGAAGGA	55 0C/600 bp	Everett *et al.* (2005)
	23SIGR	TGGCTCATCATGCAAAAGGCA		

agarose gel stained with ethidium bromide and photographed using Syngene Gel documentation system. The amplified fragments were purified from agarose gel using GeneJet Gel purification kit (Fermentas, Lithiuania) and sequenced directly to confirm the identity of the endosymbiont.

RESULTS AND DISCUSSION

PCR amplified a fragment of 200 bp from all whitefly samples collected from different crops in different region of Oman confirming the presence of only the B biotype in Oman (Table 2). None of the whitefly populations collected contained the Q biotype, however, some samples did not show specific PCR amplified fragments for B or Q biotypes indicating that they may be other biotypes or a failure of the PCR reaction (Table 2). These results are similar to those reported by Chiel *et al.* (2007) who have reported the dominance of the B biotype on tomato, squash, cucumber, pepper and eggplant and Q biotype on cotton and sage. The *Bemisia tabaci* biotype B known to colonize new host plants including the members of the family *Solanaceae*, *Compositae*, *Brassicaceae* and *Malvaceae*. This study showed that biotype B exhibits unique ability to disperse for long distances infesting different crops throughout the country. The possible explanation for the presence of only B biotype in Oman could be their well adaptation on vegetable crops and transfer of plant materials from one region to other within the country with absence of internal quarantine inspection.

All B biotype population found to be associated with secondary symbiont, *Hamiltonella* sp with exception that no secondary symbiont was detected in whiteflies collected from crops grown in Suwaiq area (Table 2). Infections with secondary symbionts is known to vary within or between

Table 2: Collection and identification of whitefly biotypes (B & Q) and presence (+) or absence (-) of secondary symbionts from crops grown in different regions of Oman

						Biotyoe		Secondary symbiont presence			
Region	Location	Collection year	Farms visited	Plant species	n*	B	Q	H	A	W	R
Musandam	Khasab	2011	2	Tomato	20	16	-	+	-	-	-
Musandam	Dibba	2011	2	Tomato	20	20	-	+	-	-	-
Musandam	Madha	2011	1	Tomato	20	20	-	+	-	-	-
Al Batinah north	Shinas	2012	5	Tomato	20	20	-	+	-	-	-
Al Batinah north	Sohar	2012	4	Tomato	20	20	-	+	-	-	-
Al Batinah north	Suwaiq	2012	4	Tomato	20	14	-	-	-	-	-
Al Batinah north	Musanah	2012	3	Tomato	20	19	-	+	-	-	-
		2012	2	Eggplant	16	13					
		2012	2	Melons	16	16					
Al Batinah south	Barka	2012	7	Tomato	20	20	-	+	-	-	-
Al Batinah south	Muscat	2012	3	Tomato	20	20	-	+	-	-	-
		2012	1	Petunia	20	20					
Al Bureimi	Bureimi	2012	3	Tomato	20	20					
Al Dakhliya	Izki	2012	4	Cucumber	20	8	-	+	-	-	-
Al Sharqiah	Sur	2012	4	Tomato	20	17	-	+	-	-	-
Al Sharqiah	Al-Kamil	2012	2	Tomato	20	20					
		2012	1	Cucumber	20	14					
Dhofar	Thumrait	2012	2	Pepper	20	20	-	+	-	-	-
		2012	1	Eggplant	20	20					
		2012	2	Pepper	20	20	-	+	-	-	-
Dhofar	Taqah	2012	2	Pepper	20	18	-	+	-	-	-
Dhofar	Salalah	2012	2	Squash	20	19	-	+	-	-	-
			1	Cabbage	20	10	-	+	-	-	-

n* = Total number of whiteflies tested from each population, H = *Hamiltonella*, A= *Arsenophonus*, W = *Wolbachia*, R= *Rickettsia*

populations since their presence is not essential for the insect. Zchori-Fien and Brown (2002) found that 40% of *Hamiltonella* associated with *B. tabaci* populations. The results reported in this study are similar to Sandstrom *et al.* (2001) who found that 100% *Hamiltonella* with *B. biotype*. Such high incidence could possibly be related to an obligatory or mutualistic relationship between *Hamiltonella* and B biotypes. This symbiont may also be involved in host physiological disorder caused by the B biotype complex (Thao and Baumann, 2004; Chiel *et al.*, 2007).

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