

# Indigenous and introduced species of the *Bemisia tabaci* complex in sweet potato crops from Argentina

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## SUMMARY

Sweet potato (*Ipomoea batatas* (L.) Lam) is one of the most important crops worldwide. Recently, the appearance of severe viral symptoms has been observed in sweet potato crops in the pampas region of Argentina and both begomovirus and crinivirus, exclusively transmitted by whiteflies, have been identified. The aim of this study was to identify *B. tabaci* species from sweet potato crops in Colonia Caroya by analysing mitochondrial cytochrome c oxidase subunit I (mtCOI) sequences. Two previously described haplotypes were identified: New World2 (indigenous species) and MEAM1 (introduced species). The results indicate the presence of both species, which are potential vectors of begomovirus and crinivirus in Argentina.

**Keywords:** whitefly, mitochondrial cytochrome c oxidase subunit I, *Ipomoea batatas*

Alemandri, V.; J.A. Martino, L. Di Feo y G. Truol, 2014. Especies nativas e introducidas del complejo *Bemisia tabaci* en cultivos de batata de Argentina. Agriscientia 31 (2): 103-107

## RESUMEN

La batata (*Ipomoea batatas* (L.) Lam) es uno de los cultivos más importantes en el mundo. Recientemente se observó una severa sintomatología viral en cultivos de la región pampeana argentina, en la que están identificados begomovirus y crinivirus, ambos transmitidos exclusivamente por mosca blanca. El objetivo de este estudio fue identificar las especies de *B. tabaci* en cultivos de batata en Colonia Caroya, mediante el análisis de secuencias mitocondriales de la citocromo oxidasa subunidad I (mtCOI). Se identificaron dos haplotipos (especies crípticas) ya descriptos en el mundo: New World2 (especie nativa) y MEAM1 (especie introducida). Los resultados indican la presencia de ambas especies, las cuales son potenciales vectores de begomovirus y crinivirus en batata en Argentina.

**Palabras clave:** mosca blanca, citocromo oxidasa subunidad I, *Ipomoea batatas*

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*Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a cryptic species complex (Dinsdale *et al.*, 2010; De Barro *et al.*, 2011) that causes annual economic damages ranging from several hundred millions to billions of dollars worldwide (Oliveira *et al.*, 2001). *B. tabaci* is a vector of emerging plant viruses worldwide, including the genera begomovirus and crinivirus (Navas-Castillo *et al.*, 2011).

Until 2010, *B. tabaci* was considered a complex composed of at least 24 distinct putative species (hereon species) (Dinsdale *et al.*, 2010; De Barro *et al.*, 2011). Recently, at least 15 new species have been reported (Hu *et al.*, 2011; Alemandri *et al.*, 2012; Chowda-Reddy *et al.*, 2012; Parrella *et al.*, 2012; Firdaus *et al.*, 2013; Esterhuizen *et al.*, 2013).

Middle East-Asia Minor 1 (hereafter MEAM1 and commonly referred to as the B biotype in the literature) is known worldwide as one of the most invasive and destructive species in the complex (Perring, 2001). In Argentina, MEAM1 was first detected on horticultural crops, weeds and cotton samples (Viscarret *et al.*, 2003). Furthermore, recent studies have demonstrated the presence of an indigenous species belonging to the *B. tabaci* cryptic species complex in Argentina, a new species affecting soybean and bean crops named New World2 (Alemandri *et al.*, 2012).

Sweet potato (*Ipomoea batatas* (L.) Lam) is one of the most important crops worldwide, with great potential not only for human consumption but also for animal feeding and industrial use (Bovell-Benjamin, 2007). Argentina produced 340,105 metric tons in 2009 (USDA Economics and System, 2011), mainly in the pampas region (Buenos Aires, Córdoba and Santa Fe provinces). Recently, severe viral symptoms have been observed in this area. Previous studies suggest that more than one etiologic agent is involved in this new viral disease, including both begomovirus and crinivirus exclusively transmitted by whiteflies (Di Feo *et al.*, 2000; Rodríguez Pardina *et al.*, 2012). Although there are records of presence of whiteflies, the

species of *B. tabaci* present in this area have not been identified. The aim of this work was to identify species of *B. tabaci* present in sweet potato crops from Colonia Caroya in Argentina by analysing mitochondrial cytochrome c oxidase subunit I (mtCOI) sequences.

Adult whiteflies (*Bemisia tabaci*) were collected from five sweet potato fields in Colonia Caroya, Colón department, Córdoba province (31° 02' 02" S, 64° 04' 46" W), in the pampas region, an important sweet potato growing area in Argentina. Live adult whiteflies were collected and placed in tubes containing 100% ethanol and maintained at 4°C until DNA extraction.

Whitefly individuals were sexed, and DNA was extracted from females, following the protocol of De Barro & Driver (1997) and Truol *et al.* (2003). A total of 10 females were analysed. Females were individually homogenized in 60 µl filtered lysis buffer (10 mM Tris-HCl, pH 8, 1 mM EDTA, 0.3% Triton X-100, 60 µg/ml proteinase K) using 1.5 ml microcentrifuge tubes. The homogenate was incubated at 65 °C for 15 min and at 98 °C for 6 min. Then, it was centrifuged at 9600 rpm for 1 min and maintained at -20 °C. The primers C1-J-2195 and L2-N-3014 were used for amplification (Simon *et al.*, 1994; Frohlich *et al.*, 1999). PCR reactions were carried out in a final volume of 25 µl containing 1 U *Taq* DNA polymerase (Invitrogen), 2.5 mM MgCl<sub>2</sub>, 0.20 mM dNTP, 0.6 µM of each primer and 2 µl of DNA. The reactions were performed with a thermal cycler programmed for one cycle of 2 min at 94 °C, followed by 30 cycles (1 min at 94 °C, 1 min at 52 °C and 1 min at 72 °C) and a final 5-min extension at 72 °C. Amplification products were analysed by 1.5% agarose gel electrophoresis. The amplified products of about 800 base pairs were purified using illustra MicroSpin S-200 HR Columns (GE Healthcare, UK) and sequenced in both directions with the amplification primers using an ABI 3130XL (Applied Biosystems) automated sequencer.

A total of 438 sequences were used to identify unique haplotypes of *B. tabaci* mtCOI sequences,

which included 10 from our own whitefly collection and 428 unique haplotypes retrieved from GenBank. The latter comprised the sequences used in Firdaus *et al.* (2013) (which in turn included nine outgroups), newly proposed groups (Chowda-Reddy *et al.*, 2012; Parrella *et al.*, 2012; Esterhuizen *et al.*, 2013) and the New World2 sequences recently detected in Brazil (Marubayashi *et al.*, 2012). All the mtCOI sequences were 657 bases in length and were aligned using the CLUSTAL W Multiple alignment procedure (Thompson *et al.*, 1994) implemented in the BIOEDIT software, version 7.0.4 (Hall, 1999). A total of 428 unique *B. tabaci* mtCOI sequence haplotypes were identified from the analysis of 438 sequences using DnaSP v5 (Librado & Rozas, 2009).

Of the 10 mtCOI sequences generated in this study, two haplotypes were identified (Hap-1 and Hap-2) that belong to two species of the *B. tabaci* complex in sweet potato crops in Argentina. Hap-1 was identical to a New World2 haplotype (JF901844) previously described in bean and soybean crops in Argentina (Alemandri *et al.*, 2012). Hap-2 was identical to a MEAM1 haplotype (GU086344) previously described in USA, Dominican Republic, Trinidad and Tobago, Brazil, Spain, Italy and South Africa (Firdaus *et al.*, 2013). Because the sequences generated in this study were not new haplotypes, phylogenetic analyses or pairwise comparison of genetic distances were not performed. Haplotypes previously described and identical to the sequences presented here were correctly identified using the new criteria proposed for *B. tabaci* (Alemandri *et al.*, 2012; Firdaus *et al.*, 2013). These two whitefly species were found to co-occur in three fields (Table 1).

The analysis of mtCOI supports the existence of two species of the *B. tabaci* complex in sweet potato fields in Argentina: an indigenous species

belonging to the New World2 putative species, and an introduced species belonging to the MEAM1 putative species. This is the first report of MEAM1 in sweet potato in Argentina, and is in agreement with a previous study reporting the presence of MEAM1 in sweet potato in Cuba using information on the mtCOI gene (Muniz *et al.*, 2011). Furthermore, several species, Asia II 1 (Liu *et al.*, 2010), Asia II 6 (Ueda *et al.*, 2009), Asia II 9, Asia II 10 (Hu *et al.*, 2011), Indian Ocean (Delatte *et al.*, 2005), Uganda (Maruthi *et al.*, 2004), Mediterranean (De la Rua *et al.*, 2006) and New World (Muniz *et al.*, 2011) were found in *I. batatas* in other countries. To the best of our knowledge, this is the first report of New World2 in sweet potato from Argentina.

On the other hand, the results presented here suggest that New World2 and MEAM1 co-occurred in sweet potato under field conditions (Table 1). Similar events have been noted in Brazil, where specimens of the MEAM1 and New World2 species were found colonizing a single plant of *Glycine maxima*, or the same plot on *Euphorbia heterophylla* (Marubayashi *et al.*, 2012). Previous studies indicate that the development of a hybrid whitefly between two species such as MEAM1 and Mediterranean is unlikely (Sun *et al.*, 2011; McKenzie *et al.*, 2012). There are no records of the development of a hybrid between New World2 and MEAM1.

The identification of the two species of the *B. tabaci* complex in sweet potato crops suggests that they would be the insect vectors responsible for sweet potato begomovirus and crinivirus transmission in Argentina. The present results highlight the need to further explore the diversity of the *B. tabaci* species present in sweet potato crops in the pampas region, as well as to conduct virus transmission experiments.

**Table 1.** Populations of *Bemisia tabaci* sampled in sweet potato crops in Argentina and identified by sequencing of the mtCOI gene.

N° Field	Host plant	Province	Collection date	Sample code	Haplotype	Putative species	GenBank Accession number
1	<i>Ipomoea batatas</i>	Córdoba	2012	Bat-1	Hap-1	New World2	KM100350
1	<i>Ipomoea batatas</i>	Córdoba	2012	Bat-2	Hap-1	New World2	KM100351
2	<i>Ipomoea batatas</i>	Córdoba	2012	Bat-3	Hap-1	New World2	KM100352
2	<i>Ipomoea batatas</i>	Córdoba	2012	Bat-4	Hap-2	MEAM1	KM100358
3	<i>Ipomoea batatas</i>	Córdoba	2012	Bat-5	Hap-1	New World2	KM100353
3	<i>Ipomoea batatas</i>	Córdoba	2012	Bat-6	Hap-1	New World2	KM100354
4	<i>Ipomoea batatas</i>	Córdoba	2012	Bat-7	Hap-2	MEAM1	KM100359
4	<i>Ipomoea batatas</i>	Córdoba	2012	Bat-8	Hap-1	New World2	KM100355
5	<i>Ipomoea batatas</i>	Córdoba	2012	Bat-9	Hap-1	New World2	KM100356
5	<i>Ipomoea batatas</i>	Córdoba	2012	Bat-10	Hap-2	MEAM1	KM100357

## ACKNOWLEDGMENTS

We would like to thank INTA –Instituto Nacional de Tecnología Agropecuaria– and Fundación ArgenINTA for financial support.

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