PCR molecular identification of the fall armyworm in the Chontalpa region, Tabasco, Mexico

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ABSTRACT

Objective: To perform the first molecular characterization of the barcoding COI region of the *S. frugiperda* found in Tabasco and to test whether it can reliably identify the strain detected in the said state.

Design/Methodology/Approach: We collected *S. frugiperda* specimens using four pheromone traps placed in corn plots. Subsequently, we implemented DNA extraction, PCR with LCO-L/HCO-L primers, and the sequencing of six individuals captured in the traps.

Results: After aligning the sequences, we developed a phylogenetic tree, determining that *S. frugiperda* belongs to the rice strain (RS).

Implications: Identifying the *S. frugiperda* strain is necessary for management purposes, since different strains may require different control methods.

Findings/Conclusions: Our study revealed that the isolates from Chontalpa, Tabasco, Mexico, match the RS fall armyworm reported in other latitudes. Consequently, this is the first report to identify this strain of *S. frugiperda* in Tabasco. Our findings are relevant because this species can potentially become a pest in sugarcane- and rice-growing areas of the state.

Keywords: *Spodoptera frugiperda*, Rice, Barcoding, Pests.

Citation: Ramos-Hernández, E., Ortiz-García, C.F., Córdova-Sánchez, S., & Castellanos-Potenciano, B. P. (2023). PCR molecular identification of the fall armyworm in the Chontalpa region, Tabasco, Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i3.2369

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: September 02, 2022.
Accepted: February 27, 2023.
Published on-line: May 19, 2023.

Agro Productividad, 16(3). March. 2023. pp: 81-89.

INTRODUCTION

The genus *Spodoptera* (Guenée, 1852) (Lepidoptera: Noctuidae) comprises 30 species widely distributed in the Americas (Acosta, 2021). The genus includes four pest species of economically relevant Noctuidae that are highly polyphagous (EPPO, 2015). One of these is *Spodoptera frugiperda* (Smith, 1797), commonly called "fall armyworm" (FAW) (Casmuz *et al.*, 2010; Paredes-Sánchez *et al.*, 2021), an invasive pest species native to the Americas that has rapidly spread around the world. It has been found in Africa (2016), India (2018), China and Egypt (2019), Australia (2020), the Canary Islands in Spain, and Saudi Arabia (2021), where it has damaged corn crops (*Zea mays* L. (Poales: Poaceae)) (Paredes-Sánchez *et al.*, 2021; Varshney *et al.*, 2021).

Image by Fer Galindo at Pixabay

Spodoptera frugiperda is a highly polyphagous pest that attacks more than 350 commercial and non-commercial hosts within 76 plant families (Maruthadurai and Ramesh, 2020; Montezano *et al.*, 2018) and causes significant economic damage to host crops such as rice (*Oryza sativa* L. (Poales: Poaceae)), cotton (*Gossypium* spp. (Malvales: Malvaceae)), sorghum (Poales: Poaceae), peanut (*Arachis hypogaea* L. (Fabales: Fabaceae), and grasses (Casmuz *et al.*, 2010). *Spodoptera frugiperda* has such an ample host range partly due to the presence of two species populations —called rice strain (RS) and corn strain (CS)— with varying host plant preferences; hence their designation as "host strains" (Pashley, 1986).

Since morphological techniques are not reliable during the larval stage of the pest, other identification tools are needed. Specific polymerase chain reaction (PCR) tests constitute a reliable method to correctly identify the FAW species from the moment they hatch and during the larval stages, based on the DNA of specimens found in a given region or state.

DNA-based methods therefore provide an independent and universal identification tool applicable to all stages of life (Mahat *et al.*, 2021). For this reason, identifying invasive species through DNA barcoding is increasingly recommended and performed globally (Floyd *et al.*, 2010; Phillips *et al.*, 2019; Yousaf *et al.*, 2022). During the barcoding process, a region of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene is used to identify species of economic and ecological relevance. In the case of pests, this process contributes to the success of management programs based on the correct identification of the organism and its host (Yousaf *et al.*, 2022). This paper presents the sequencing of the proposed barcode region of the COI gene using adult individuals of *S. frugiperda* collected from corn plots. We use these COI gene data to 1) test whether the barcoding of the COI region can reliably identify *S. frugiperda* strains, and 2) report the first molecular characterization of *S. frugiperda* in the State of Tabasco.

MATERIALS AND METHODS

Insect collection

The adult *S. frugiperda* specimens were collected in corn fields of the Huimanguillo Experimental Field of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP-CEHUI) during 2021-2022. Pherocon Faw™ pheromone traps (Ferommis®) were used to capture adult males. All adult specimens were preserved in ethanol (95%) and stored at −80 °C in the phytopathology laboratory of the Colegio de Postgraduados–Campus Tabasco.

DNA extraction, amplification, and sequencing

The whole insect was used for the extraction of the DNA. Genomic DNA was extracted from adult insects according to protocol, using the cetyltrimethylammonium bromide (CTAB) insect DNA extraction method (Harrison *et al.*, 1996; Brown *et al.*, 2006). The DNA was resuspended in 30 μL of ultrapure water and stored at 4 °C. The barcoding COI region was amplified using the universal primer pair LCO1490-L (5'-GGTCWAC WAATCATAAAGATATTGG-3') and HCO2198-L (5'-TAAACTTCWGGRTGWCC AAARAATCA-3') —which are slightly modified forms obtained by Nelson *et al.* (2007) from the primers designed by Folmer *et al.* (1994). Every 25 μL of the polymerase chain

reaction (PCR) mix contained 5X reaction buffer, 50mM $MgCl_2$, 100 ng of each primer, 100 μM each of dATP, dCTP, dGTP, and dTTP (Invitrogen, USA), 0.5 U of Mango Taq^{TM} (Bioline, United Kingdom), and 50 ng of genomic DNA. PCR temperature cycles were performed in a C100 Thermal Cycler (Bio-Rad, Hercules, CA) as follows: initial denaturation cycle at 94 °C for 2 min, then 35 cycles at 94 °C for 30 min, followed by 50 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. PCR products were visualized using electrophoresis on 2.0% agarose gel stained with ethidium bromide. Sequencing was performed in both directions with amplification primers using the 3500xl Genetic Analyzer (Applied Biosystems, USA) at the Instituto de Biotecnología of the Universidad Nacional Autónoma de México.

DNA sequence analysis

Sequences were refined and manually edited using the Bioedit 7.2.5 software (Hall, 1999). To secure their identification, several searches were conducted for each sequence in the Basic Local Alignment Search Tool (BLAST) system of the National Center for Biotechnology Information (NCBI, http://www.ncbi.nl-m.nih.gov). The ClustalX 2.1 software (with its default settings) was used for sequence alignment (Thompson *et al.*, 1997). Based on the *S. frugiperda* sequences, a phylogenetic tree was developed using the Molecular Evolutionary Genetics Analysis (MEGA) XI software (Tamura *et al.*, 2021). Data were subjected to maximum likelihood (ML) analyses based on the K-2 parameter model and evaluated for bootstrap analysis with 1,000 replicates, in order to estimate the stability of the inferred subclades. The outgroup for analysis was *Bombyx mori* L. All sequences in the present study (n=6) have been submitted to GenBank. In addition, reference sequences from GenBank (Table 1) were downloaded and incorporated into the phylogenetic analysis. To classify the strain in our phylogenetic analysis, we included barcodes designated as references for RS (Accession No. U72977) and CS (Accession No. U72974) in previous studies (Maruthadurai and Ramesh, 2020; Nelly *et al.*, 2021; Sarr *et al.*, 2021).

RESULTS AND DISCUSSION

Specimens

Forty-five adult males were collected from four traps located on the perimeter of corn plots between December 14, 2021, and March 15, 2022. Since the specimens sequenced in this study come from traps placed on the edges of corn plots, we do not have detailed data on the preferred host plants of *S. frugiperda*. However, larvae were indeed observed in corn plants at INIFAP-CEHUI.

Molecular identification and analysis

The *S. frugiperda* barcoding region COI was easy to amplify and sequence. According to other researches, sequences were easily aligned easily, given the absence of insertions and deletions (Hebert *et al.*, 2003). The mitochondrial DNA cytochrome c oxidase subunit 1 (COI) gene is commonly used to identify biotypes and study insect genetics (Kasambala Donga and Meadow, 2018). Cock *et al.* (2017), Maruthadurai and Ramesh (2020), and Nelly *et al.* (2021) recently observed that the amplifications of this barcoding region for

the molecular characterization of *S. frugiperda* produce a single, thick, and clear band with amplicons of ±700 bp.

The six samples selected for barcode analysis generated a visible PCR product of the expected size (approx. 680 bp) representing *S. frugiperda*. These PCR products generated usable DNA sequences. All six sequences in this study were submitted to the GenBank databases on March 12, 2022. The sequences were identified as *S. frugiperda*, after nBLAST analyses of the data of these COI sequences were carried out in the NCBI GenBank database. Our findings are supported by a 100% similarity in sequence and coverage data. The sequences were trimmed to 643 bp and used in the phylogenetic analysis (Figure 1). For comparison purposes, 26 GenBank sequences were downloaded, including *S. frugiperda*

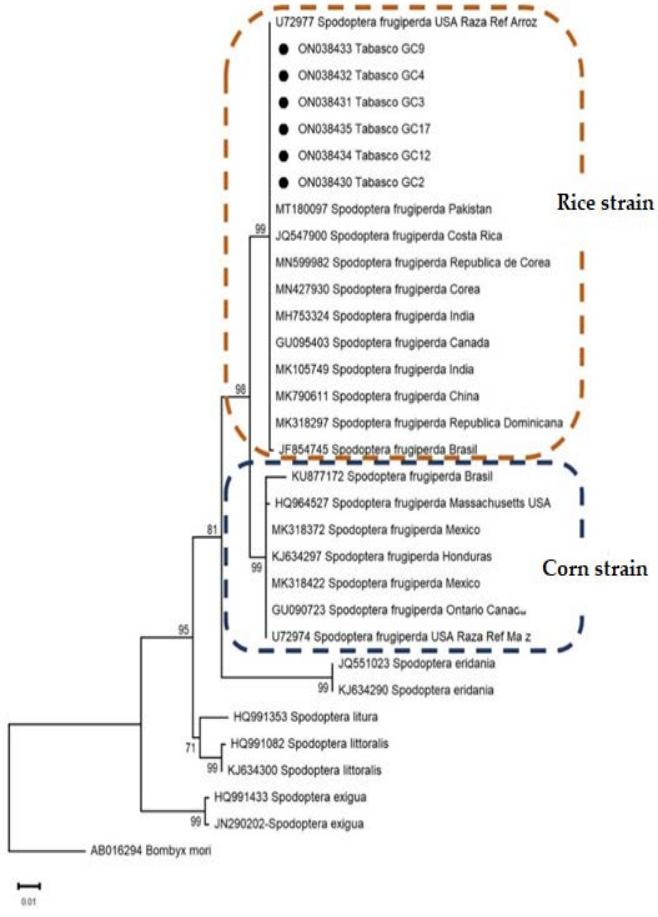

Figure 1. Phylogenetic tree of *Spodoptera frugiperda* in Tabasco, Mexico. Cytochrome c oxidase subunit 1 (COI) sequences from five species of Lepidoptera: Noctuidae were used to developed the tree in Figure 1, while *Bombyx mori* was used as an outgroup. The maximum likelihood method (ML) and Kimura's two-parameter model (K-2) (Kimura, 1980) was used to infer the evolutionary history. The tree with the highest log probability (−1,832.16) is shown here. The percentage of trees with clustered associated taxa can be found next to the branches. The first trees were developed in order to conduct the heuristic search, applying the Neighbor-Joining method to a pairwise distance matrix, calculated with the maximum composite likelihood (MCL) approach. The numbers in the nodes indicate bootstrap values (>50%, 1,000 replicates). GenBank accession numbers are shown before the namem of each species. The bullet points indicate the samples collected in Tabasco for this study. The scale bar measures substitutions per site. The Clustal W software was used to align the nucleotide sequences and MEGA 11 was used to perform the evolutionary analyses (Tamura *et al.*, 2021).

strains from corn (n=7) and rice (n=11). *S. litoralis* (Boisduval, 1833) (n=2), *S. eridania* (Cramer, 1782) (n=2), *S. exigua* (Hübner, 1808) (n=2), *S. litura* (Fabricius, 1775) (n=1), and *Bombyx mori* (Linnaeus, 1758) (n=1) were selected as outgroup (Table 1).

The sequences of the barcoding COI region of *S. frugiperda t*rapped at INIFAP-CEHUI clearly group with the reference sequences of the rice strain (U72977) (Figure 1).

The maximum likelihood analysis supported the results of Hebert *et al.* (2010), who considered that COI gene barcoding was an effective identification tool. *Spodoptera frugiperda*

Table 1. Specimen data and GenBank accession numbers used in this study.

Species	Reference	GenBank	Gene	Country
Bombyx mori	Kim *et al.*, (2000)	AB016294	COI	
Spodoptera exigua	Zahiri *et al.*, (2017)	JN290202	COI	U.S.
Spodoptera exigua	Zahiri *et al.*, (2017)	HQ991433	COI	Pakistan
Spodoptera frugiperda	This study	ON038430	COI	Mexico
Spodoptera frugiperda	This study	ON038431	COI	Mexico
Spodoptera frugiperda	This study	ON038432	COI	Mexico
Spodoptera frugiperda	This study	ON038433	COI	Mexico
Spodoptera frugiperda	This study	ON038434	COI	Mexico
Spodoptera frugiperda	This study	ON038435	COI	Mexico
Spodoptera littoralis	Ashfaq *et al.*, (2017)	HQ991082	COI	Pakistan
Spodoptera littoralis	van de Vossenberg & van der Straten, (2014)	KJ634300	COI	Zimbabwe
Spodoptera litura	Ashfaq *et al.*, (2017)	HQ991353	COI	Pakistan
Spodoptera eridania	Zahiri *et al.*, (2017)	JQ551023	COI	Costa Rica
Spodoptera eridania	van de Vossenberg & van der Straten, (2014)	KJ634290	COI	Surinam
Spodoptera frugiperda	Maas & Sanjur, (1996)	U72974	COI	U.S.
Spodoptera frugiperda	van de Vossenberg & van der Straten, (2014)	KJ634297	COI	Honduras
Spodoptera frugiperda	Gilligan *et al.*, (2019)	MK318422	COI	Mexico
Spodoptera frugiperda	Ratnasingham & Hebert, (2013)	GU090723	COI	Canada
Spodoptera frugiperda	Zahiri *et al.*, (2017)	HQ964527	COI	U.S.
Spodoptera frugiperda	Gilligan *et al.*, (2019)	MK318372	COI	Mexico
Spodoptera frugiperda	-	KU877172	Mitogenome	Brazil
Spodoptera frugiperda	-	JF854745	COI	Brazil
Spodoptera frugiperda	Gilligan *et al.*, (2019)	MK318297	COI	Dominican Republic
Spodoptera frugiperda	-	MK105749	COI	India
Spodoptera frugiperda	Hebert *et al.*, (2010)	GU095403	COI	Canada
Spodoptera frugiperda		MH753324	COI	India
Spodoptera frugiperda	Jing *et al.*, (2020)	MK790611	COI	China
Spodoptera frugiperda	-	MN427930	Mitogenome	Korea
Spodoptera frugiperda	-	MT180097	COI	Pakistan
Spodoptera frugiperda	-	MN599982	Mitogenome	South Korea
Spodoptera frugiperda	-	JQ547900	COI	Costa Rica
Spodoptera frugiperda	Maas & Sanjur, (1996)	U72977		U.S.

—which encompasses the "rice" and "corn" strains, based on host plant preferences— does not show any morphological differences (Nagoshi *et al.*, 2015; Pashley, 1986).

Therefore, in this study molecular identification methods were more accurate than morphological identification (Jing *et al.*, 2020). Although the strains are morphologically indistinguishable, they have different genetic markers, with polymorphisms in the mitochondrial COI gene among the best characterized. Nagoshi *et al.* (2015) mention a deficiency in the identification of the distribution pattern of the FAW CS in Central America and Mexico —which is far less significant regarding the RS.

Through the phylogenetic comparison of the COI region, the six sequences used in this study are grouped into the *S. frugiperda* RS clade (Figure 1); these results differ from those previously reported in Mexico. An earlier study used two segments of the mitochondrial COI gene to confirm that populations from four FAW colonies in Mexico (Durango, Sinaloa, Tamaulipas, and Chiapas) belong to the *S. frugiperda* CS clade (Nagoshi *et al.*, 2015).

The barcodes obtained from our samples were compared with public barcodes in GenBank, revealing that the isolates from Chontalpa, Tabasco, genetically align with FAW strains from other latitudes and with the reference RS GenBank No. U72977. The alignment of the sequences used in this study shows a 100% match with those reported for the RS in India (Swamy *et al.*, 2018); the CS in China (Jing *et al.*, 2020), Korea (Kim *et al.*, 2021), and Pakistan (Lalramnghaki *et al.*, 2021); sorghum in India (GenBank No. MH753324); sugarcane in Malawi (Kasambala Donga and Meadow, 2018); and *chili* spp. in the Dominican Republic (Gilligan *et al.*, 2019), Costa Rica (GenBank No. JQ547900), and Canada (GenBank No. GU095403).

This is the first genetic study characterizing the FAW in Tabasco by using a region of the COI gene. Strain identification of *S. frugiperda* is necessary for management purposes, as each strain may require a different control method. Moreover, some *S. frugiperda* populations show extraordinarily high resistance to insecticides such as pyrethroids, organophosphates, and diamides (Kim *et al.*, 2021).

Having established the presence of the *S. frugiperda* RS in the Chontalpa area of Tabasco, further studies are required to identify the *S. frugiperda* CS, in order to achieve an effective and efficient control. The latter must consider these species' resistance to insecticides and the distribution of both strains. The two strains breed continuously from the southern US to northern Argentina, and both are found further north and further south during summer and fall, since they are seasonally breeding migrant populations, but cannot tolerate <0 temperatures (Cock *et al.*, 2017).

The Food and Agriculture Organization (FAO) of the United Nations recommends using pheromone traps to determine the incidence and severity of *S. frugiperda* (Prasanna *et al.*, 2018). Accurate identification of pest species is crucial to the effectiveness of pheromone traps as a monitoring tool. However, Unbehend *et al.* (2014) report that males of the corn and rice strains had diverse responses to different combinations of synthetic pheromones in various geographic regions.

The RS seems to colonize corn, sweet corn, and sorghum (Swamy *et al.*, 2018). However, although the strains are named after their preferred host plants, there is no certainty as

to the host specificity of each one. Both the RS and the CS feed on corn during the same cultivation period (Nagoshi *et al.*, 2007), a phenomenon which was already reported by Pashley (1986), who differentiated both strains by food preference, although not the specificity of the host plant.

Therefore, DNA barcoding will facilitate the identification of pests in the country and support taxonomic work where necessary —*i.e.*, for species requiring specialized studies to enable identification and lacking a reference barcode, as well as for species that require differentiation as a consequence of their morphologically similar taxa.

CONCLUSIONS

The DNA barcode approach (barcoding) facilitated the identification of the *S. frugiperda* RS in southern Mexico. Confirmation of the presence of RS in the study area is of the utmost importance, since this strain of *S. frugiperda* can infest sugarcane, rice, and corn crops. Considering the year-round availability of host plants and the voracious nature of *S. frugiperda*, this species can potentially become a dangerous pest in sugarcane- and rice-growing areas of Tabasco, if no effective measures are taken to control its spread.

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