SHORT COMMUNICATION

HOST STRAIN IDENTIFICATION OF *Spodoptera frugiperda* (J.E. SMITH) FROM SERIAN AND KOTA SAMARAHAN, SARAWAK, MALAYSIA

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ABSTRACT

Spodoptera frugiperda (J.E. Smith) is one of the most invasive pests around the world, causing significant economic losses to corn and rice farmers. In September 2019, the Department of Agriculture (DOA), Sarawak, Malaysia has confirmed the presence of *S. frugiperda* in corn fields of Kuching, Sarawak. This study aimed to identify the host strain of 15 *S. frugiperda* isolates from Serian and Kota Samarahan, Sarawak, using the mitochondrial cytochrome oxidase subunit 1 (*CO1*) gene marker. Based on the *CO1* sequence, corn and rice strain of *S. frugiperda* were identified among the 15 *S. frugiperda* isolates. Based on the migration pattern of *S. frugiperda*, it appears that only corn strain migrated to Asian countries; thus, this study proposes that interstrain hybrid may be presence in corn fields of Serian and Kota Samarahan. *CO1* gene alone is ineffective to identify the host strain of *S. frugiperda*. Therefore, further study should include the nuclear sex-linked triosephosphate isomerase (*TPI*) gene sequence in addition to *CO1* gene sequence for more conclusive host strain identification of *S. frugiperda*.

Keywords: *CO1*, genetic diversity, host strain, *Spodoptera frugiperda*

ABSTRAK

Spodoptera frugiperda (J.E. Smith) merupakan salah satu serangga perosak invasif di dunia yang boleh menyebabkan kerugian ekonomi yang ketara kepada penanaman jagung dan padi. Pada September 2019, Jabatan Pertanian Sarawak (DOA), Malaysia telah mengesahkan kehadiran *S. frugiperda* di ladang jagung di Kuching, Sarawak. Kajian ini bertujuan untuk mengenal pasti strain perumah daripada 15 pencilan *S. frugiperda* di kawasan Serian dan Kota Samarahan, Sarawak, dengan menggunakan penanda (*CO1*) mitokondria, sitokrom oksidase subunit 1. Merujuk jujukan *CO1*, strain jagung dan padi *S. frugiperda* telah dikenalpasti daripada 15 pencilan *S. frugiperda* tersebut. Berdasarkan corak migrasi *S. frugiperda*, hanya strain jagung yang berhijrah ke negara Asia. Oleh itu, kajian ini mencadangkan bahawa kemungkinan strain *S. frugiperda* di ladang jagung Serian dan Kota Samarahan ialah strain hibrid di antara strain jagung dan padi. Pengesahan perumah strain *S. frugiperda* dengan menggunakan gen *CO1* sahaja adalah tidak berkesan. Oleh itu, kajian selanjutnya perlu menggunakan jujukan gen *nuclear sex-linked triosephosphate isomerase* (*TPI*) sebagai tambahan kepada jujukan gen *CO1* untuk pengenalpastian perumah strain yang lebih konklusif bagi *S. frugiperda*.

Kata kunci: *CO1*, kepelbagaian genetik, strain perumah *Spodoptera frugiperda*

Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a noctuid pest that could migrate thousands of kilometres away from its original habitat (Nagoshi et al. 2019). *Spodoptera frugiperda* is a polyphagous species that feeds on more than 353 species of host plants from 76 plant families (Montezano et al. 2018). Dumas et al. (2015) discovered two main strains of *S. frugiperda* that are morphologically indistinguishable and may differ in mating behaviour, pheromone compositions, and show development variability according to the strain. These two strains of *S. frugiperda* are corn-strain (C-strain) and rice-strain (R-strain). C-strain feeds on corn, cotton, and sorghum while R-strain feeds on rice and various pasture grasses, preferentially (Juárez et al. 2012). The identification of *S. frugiperda* strain can be based on the sex-linked triosephosphate isomerase (*TPI*) and mitochondrial cytochrome oxidase subunit 1 (*CO1*) gene sequences (Nagoshi 2012).

In early 2019, C-strain of *S. frugiperda* has migrated to Perlis, Malaysia. *Spodoptera frugiperda* was then reported to be in Borneo in December 2019, involving Sarawak (Malaysia) (IPPC 2019), Sabah (Personal Communication, Department of Agriculture Sabah), Brunei (Ministry of Primary Resources and Tourism of Brunei 2021) and Kalimantan (Maharani et al., 2021). The origin of *S. frugiperda,* which migrated to Borneo is unknown and the strain of *S. frugiperda* in Sarawak has not been identified. Therefore, the objective of this project was to identify the strain of *S. frugiperda* migrated to Sarawak based on *CO1* gene sequence. It is crucial to research *S. frugiperda* in Sarawak in order to be better prepared to combat *S. frugiperda* in Sarawak.

A total of 15 *S. frugiperda* isolates were collected, with eight isolates coming from Serian division and seven isolates from Kota Samarahan. Their *CO1* gene was amplified using primer

pair LCO1490 (5' GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5' TAAACTTCAGGGTGACCAAAAAATCA-3') following Acharya et al. (2021). The 15 *CO1* gene sequences were analysed with Basic Local Alignment Search Tool (BLAST) against the sequences in National Center for Biotechnology Information (NCBI) for species identification. The BLAST result showed that all the 15 *S. frugiperda* samples were 99 - 100% identical to *S. frugiperda* (Table 1).

Table 1. BLAST result for *CO1* gene sequences isolated from 15 *Spodoptera frugiperda* of Sarawak, Malaysia

Isolate Code	Sample	Location	Accession Number (First hit) from NCBI BLAST	Similarity Percentage	Max Identity	E-value	Accession Number
SerL1	Larva	Serian	MH899611.1	100	1206	0	OR002052
SerL2	Larva	Serian	MW876208.1	100	1205	0	OR002053
SerL3	Larva	Serian	MN541574.1	99.7	1227	0	OR002054
SerL4	Larva	Serian	MN541574.1	99.7	1229	0	OR002055
SerA6	Adult	Serian	MN541574.1	100	1201	0	OR002056
SerA7	Adult	Serian	MN541574.1	100	1216	0	OR002057
SerA8	Adult	Serian	MW876208.1	100	1221	0	OR002058
SerA9	Adult	Serian	MN541574.1	100	1223	0	OR002059
KSL1	Larva	Kota Samarahan	MW876208.1	100	1206	0	OR002060
KSL2	Larva	Kota Samarahan	MW876208.1	100	1206	0	OR002061
KSL3	Larva	Kota Samarahan	MH899611.1	100	1206	0	OR002062
KSL4	Larva	Kota Samarahan	MW876208.1	100	1206	0	OR002063
KSL5	Larva	Kota Samarahan	MH899611.1	100	1205	0	OR002064
KSL6	Larva	Kota Samarahan	MN541574.1	100	1218	0	OR002065
KSL7	Larva	Kota Samarahan	MN541574.1	100	1223	0	OR002066

For host strain identification, a sequence fragment of 597 bp, from 72 to 668 bp of *CO1* gene (based on MN599981) was used. According to Acharya et al. (2021), 10 single nucleotide polymorphic sites can be used for *S. frugiperda* strain identification. The polymorphic base sites were designated with a code "mCO1" followed by base pair number, e.g.: mCO1xxx. The 10 polymorphic sites of *CO1* gene sequence reported by Acharya et al. (2021), and one additional polymorphic site, reported by Herlinda et al. (2021), had classified the 15 *S. frugiperda* isolates into C- and R-strain (Table 2). Twelve isolates were C-strain while the remaining three isolates were R-strain. In the corn fields of Serian and Kota Samarahan, both strains were present.

Table 2. Result of 15 *Spodoptera frugiperda* samples with eleven polymorphic sites by using
        CO1 gene   accession MN599981.1 as reference

Isolate code	Nucleotide											Strain identification
	72	117	171	207	258	489*	564	570	600	634	663	
MN599981.1[!]	G	G	T	T	C	T	T	C	C	T	T	Corn strain
SerL2	G	G	T	T	C	T	T	C	C	T	T	Corn strain
SerL3	G	G	T	T	C	T	T	C	C	T	T	Corn strain
SerL4	G	G	T	T	C	T	T	C	C	T	T	Corn strain
SerA6	G	G	T	T	C	T	T	C	C	T	T	Corn strain
SerA7	G	G	T	T	C	T	T	C	C	T	T	Corn strain
SerA8	G	G	T	T	C	T	T	C	C	T	T	Corn strain
SerA9	G	G	T	T	C	T	T	C	C	T	T	Corn strain
KSL1	G	G	T	T	C	T	T	C	C	T	T	Corn strain
KSL2	G	G	T	T	C	T	T	C	C	T	T	Corn strain
KSL4	G	G	T	T	C	T	T	C	C	T	T	Corn strain
KSL6	G	G	T	T	C	T	T	C	C	T	T	Corn strain
KSL7	G	G	T	T	C	T	T	C	C	T	T	Corn strain
MN599981.1[!]	A	A	C	A	T	C	C	T	T	C	A	Rice strain
SerL1	A	A	C	A	T	C	C	T	T	C	A	Rice strain
KSL3	A	A	C	A	T	C	C	T	T	C	A	Rice strain
KSL5	A	A	C	A	T	C	C	T	T	C	A	Rice strain

*Indicates the additional polymorphic site which able to identify *S. frugiperda* into C- and R-strain, [!] MN599981 was used as reference sequence based on Acharya et al. (2021).

The presence of C- and R-strains in a corn field may complicate the management of *S. frugiperda* because the strains differ in their tolerance to pesticides such as the well-known *Bacillus thuringiensis* Berliner (Bt) toxins (Adamczyk 1997). According to a recent study, the C-strain of *S. frugiperda* is more resistant to Bt toxins than the R-strain (Ingber 2018). Furthermore, the presence of R- strain in the corn fields of Serian and Kota Samarahan may pose a risk to the nearby rice fields in Serian and Kota Samarahan. According to Ali et al. (2018), *S. frugiperda* will infest a nearby rice field and turn the rice plant into a host after initially infesting a corn field, their primary host.

Based on previous reports, from Africa to India, China, Myanmar, Vietnam, Thailand and now Malaysia, the reported occurrence of *S. frugiperda* was found to be attacking corn field first instead of other crops (Day et al. 2017; Hang et al. 2020; IPPC 2019; Mallapur 2018; Sun et al. 2021; Yee et al. 2019). Based on this observation, only corn strain is expected to be migrated from Africa to Southeast Asia. Therefore, the identification of R-strain *S. frugiperda* in Serian and Kota Samarahan's corn fields may have been a misidentification. According to Nagoshi et al. (2012), *S. frugiperda* isolate identified as R-strain using *CO1* gene may be identified as C-strain by using *TPI* gene. Based on Juárez et al. (2012), this is because hybridization may occur between female R-strain and male C-strain, thus will show R-strain haplotype based on maternally inherited *CO1* gene (Nagoshi et al. 2012). Strain identification

using *TPI* gene requires the use of male sample. Unfortunately, male sample was not available for this study. Strain identification using both *CO1* and *TPI* gene sequences together could provide a better mean not only for strain identification, but also to identify potential instances of interstrain hybridization (Nagoshi et al. 2010). These two genes should be used together for future strain identification of *S. frugiperda* in Sarawak.

The 15 *CO1* gene sequences from this study (597 bp) were then aligned with 41 *CO1* gene sequences of *S. frugiperda* selected from NCBI using ClustalW (MEGAX). The 41 *CO1* gene sequences were from Acharya et al. (2021) and Herlinda et al. (2021), where the gene sequences were from 41 *S. frugiperda* isolates infesting corn plant. A phylogenetic tree for 56 sequences was constructed by using maximum likelihood method along with *Spodoptera litura* (Accession No. MN457695.1) as an outgroup. One thousand bootstrap replicates were used to test the phylogeny with Tamura 3-parameter model and gamma distribution rate of variation (Felsenstein 1985).

The phylogenetic tree (Figure 1) shows that the 56 *CO1* gene sequences of *S. frugiperda* were divided into two clades (I and II). Clade I consists of 39 *CO1* gene sequences of *S. frugiperda* identified as R-strain, while clade II consists of 17 sequences of C-strain. The R-strain isolates identified in this study were clustering with the R-strain isolates of Acharya et al. (2021) and Herlinda et al. (2021). This is also true for C-strain isolates. Although the two strains are separated into two distinct clusters, hybrid strain of C- and R-strain exist in the field (Nagoshi et al. 2010). The presents of hybrid strain may complicate the curbing of *S. frugiperda* using pesticides (Ingber et al. 2018). Besides that, the behaviour of hybrid strain is yet to be determined. To summarise, the 15 Sarawak *S. frugiperda* isolates are divided into C- and R-strains based on mitochondrial *CO1* gene. In the future, both *CO1* and TPI gene sequences will be used together to improve strain identification while also identifying potential instances of interstrain hybridization.
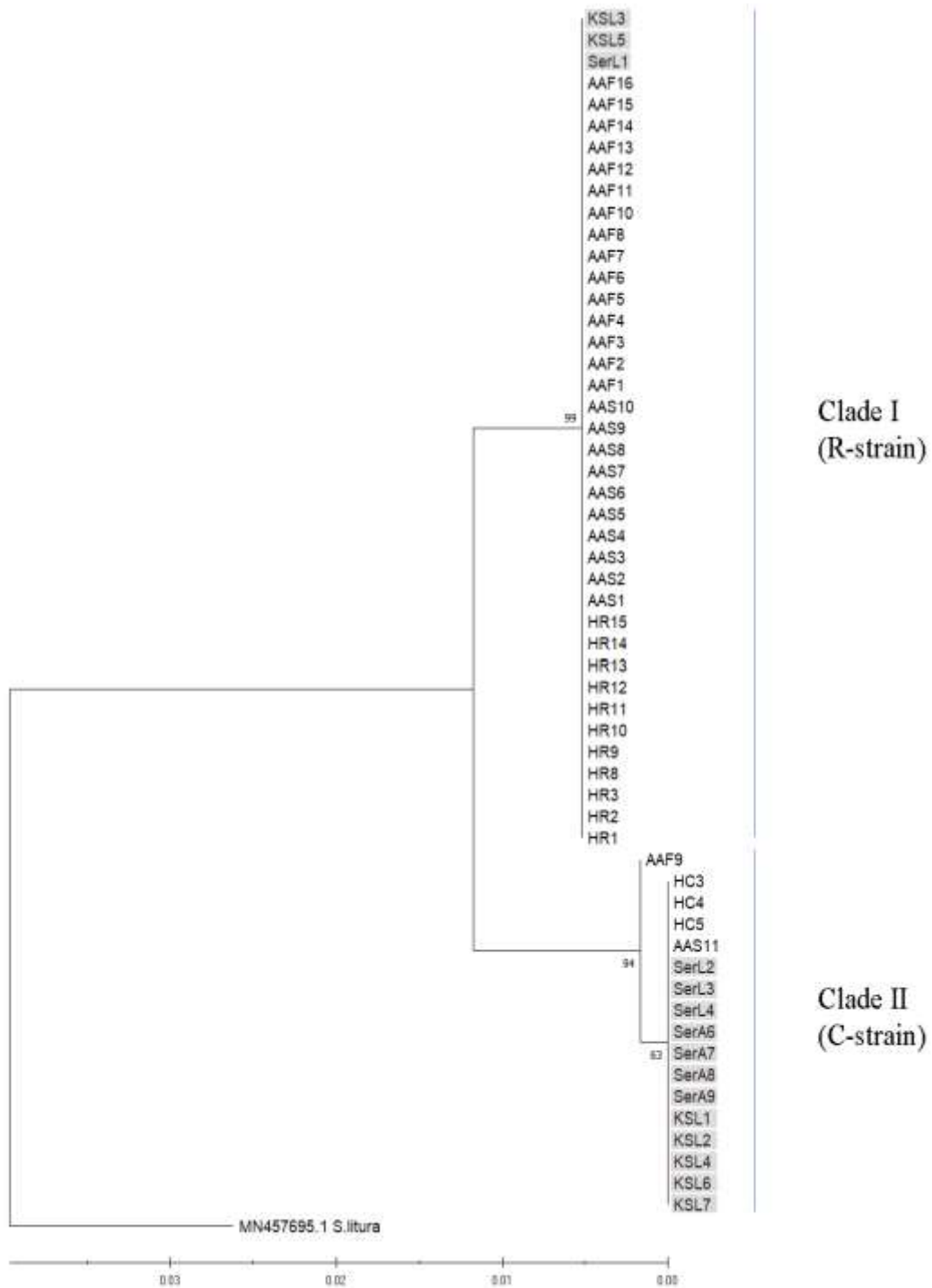
Figure 1.   A maximum likelihood phylogenetic tree (1000X bootstrap; Tamura 3-parameter model) using 56 *CO1* gene sequences of *S. frugiperda* from this study, Acharya et al. (2021) and Herlinda et al. (2021). *Spodoptera litura* (Accession NoMN457695) was used as an outgroup. Shaded isolate number indicates the 15 isolates from this study

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AUTHORS DECLARATIONS

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Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Declarations

No ethical issue required for this research.

Data Availability Statement

All sequences were made available in National Center for Biotechnology Information (NCBI)

Authors' Contributions

CWK and FKSY conceived this research and designed experiments; All authors performed experiment and analysis. CWK and FKSY wrote the paper. All authors participated in revision of it and approved the final manuscript.

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